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# I THE METABOLIC PROPERTIES OF PLUTONIUM AND ALLIED MATERIALS

J. G. Hamilton

Project 48A-I

## Radioautographic Studies

Using the costochondral junction of the rat, short term experiments with radio-strontium have been completed. The time intervals studied were 5, 10, 20, and 40 minutes. Within five minutes, radio-strontium is found on the bony rib surfaces (in the region of the periosteum and endosteum), and also at the junction of perichondrium and calcified cartilage. At forty minutes, strontium is still on the bone surfaces, but has begun to penetrate the calcified cartilage. After one hour, it is apparently beginning to penetrate the cortical bone, slightly, and is definitely present in the calcified cartilage.

Experiments at 72 hours showed Sr definitely in the compact bone and in the calcified cartilage as well. After 1 hour, in phosphorus deficient rats, Sr was beginning to penetrate the cortical bone in a manner similar to the normal controls, and by 72 hours was definitely in the compact bone. Strontium was absent from osteoid tissue in the phosphorus deficient animals. Uptake by calcified cartilage was similar to controls.

A study of the deposition of element 61 in the costochondral junction demonstrated the presence of this element on the bony rib surfaces (in the region of the periosteum and endosteum) and its absence from the cortical bone. Since this bone is quite avascular, one would not expect to find a spotty pattern as is found in the compact bone of the femur. Some 61 was found at the junction of the calcified cartilage and the perichondrium.

An attempt is underway to specifically locate the site of deposition by floating the sections on to special emulsions.

The experiments with neodymium show a femur radioautographic pattern similar to that of element 61, i.e. a spotty distribution in the cortical bone. Neodymium radioautographs of the costochondral junction were also like element 61. Radioautographs of tin distribution in the femur showed a surface depostion on the shaft and a fairly heavy deposition under the cartilage plate. The concentration of tin in the bone was quite low and the autographs were set up for a long period of time and gave rather poor resolution.

Experiments to study praseodymium in the femur and the costochondral junction, plutonium in scar tissue, and plutonium in the costochondral junction are still in the process of being completed.

## Tracer Studies

The major activities of the tracer group have been concerned with the metabolism of $Nd^{147}$, $Pr^{143}$, $Sr^{163}$, and $Ga^{67}$. The studies on $Pr^{143}$ are not yet in final form and will not be presented here.

Neodymium 147. This lanthanide rare earth, resulting from fission, was given to rats intramuscularly as 1) carrier-free material, 2) carrier-free material complexed with citrate, 3) carrier-free $Nd^{147}$ complexed with oxalate, 4) with added carrier neodymium, and 5) with added carrier praseodymium. In addition to this, studies were made with $Nd^{147}$ administered orally and intravenously. The intramuscular studies were conducted at 1, 4, 16, and 32 days after administration of $Pr^{147}$.

Neodymium 147 (Carrier-free). $Nd^{147}$ is not readily absorbed from the injection site. Following intramuscular injection, 70.5, 53.2, 38.1, and 33.9 percent of the material administered remained at the site of injection 1, 4, 16, and 32 days following administration. The distribution of the neodymium absorbed follows the pattern of the other rare earths. The material remaining in the body is found primarily in the skeleton. Appreciable amounts of activity were found in such soft tissue as liver and kidney. The major amount of Nd 147 eliminated was found in the feces. The data for all of the time periods studied are summarized in Table I.

Neodymium 147 (Carrier-Free) Complexed with Citrate. Two milligrams of sodium citrate were administered with the $Nd^{147}$. This greatly enhanced the absorption of $Nd^{147}$. At 1, 4, 16 and 32 days after intramuscular administration, only 4.5, 3.2, 1.7, and 1.7 percent remained at the injection site. When compared to the uncomplexed carrier-free studies, it can be seen that the absorption was enhanced twenty-fold. $Nd^{147}$ complexed with citrate is not handled differently in the body when compared to the uncomplexed material. The major organ of retnetion was the skeleton, fairly large amounts remaining in the liver and kidney up to 32 days following administration. The majority of the material was excreted in the feces. The data are summarized in Table II.

Neodymium 147 (Carrier-free) Complexed with Oxalate. Oxalic acid does not enhance the absorption of $Nd^{147}$ from an intramuscular injection site when used as a complexing agent. Consequently, the $Nd^{147}$ complexed with oxalic acid remained at the injection site in a manner similar to the uncomplexed carrier-free material. At 1, 4, 16 and 32 days after injection, 88.0, 68.0, 43.0 and 31.0 percent was unabsorbed. The distribution in the tissues of the $Nd^{147}$ absorbed was similar to the carrier-free $Nd^{147}$. The excretory route was similar as well. These data are summarized in Table III.

Neodymium 147 with Added Isotopic Neodymium (1/2 mgm. per rat). The administration of carrier-neodymium with radio-neodymium reduces absorption when compared to carrier-free $Nd^{147}$. Thus, 1, 4, 16 and 32 days after intramuscular administration, 86.9, 89.2, 99., and 97. percent of the administered material remained at the injection site.

Neodymium 147 with Carrier Praseodymium. In addition to the studies conducted with neodymium carrier, the effect of another rare earth of the lanthanide series was tried in order to compare its effect upon the metabolism of neodymium. Praseodymium was chosen. When one-half milligram of Pr was injected with the $Nd^{147}$, absorption from the injection site was greatly reduced. 98, 98, 92 and 98 percent of the activity

TABLE I

DISTRIBUTION OF CARRIER-FREE $Nd^{147}$ IN RATS FOLLOWING INTRAMUSCULAR ADMINISTRATION. VALUES CORRECTED FOR RECOVERY AND ABSORPTION ARE GIVEN AS PERCENT OF DOSE IN TISSUES LISTED

Time After Injection

| | 1 Day | | 4 Days | | 16 Days | | 32 Days | |
|---|---|---|---|---|---|---|---|---|
| | % per organ | % per gram | % per organ | % per gram | % per organ | % per gram | % per organ | % per gram |
| Heart | .16 | .16 | .10 | .12 | .07 | .07 | .06 | .05 |
| Lungs | .36 | .16 | .24 | .10 | .15 | .07 | .16 | .07 |
| Spleen | .23 | .22 | .20 | .19 | .09 | .09 | .16 | .18 |
| Blood | .64 | .04 | .11 | .01 | .01 | .01 | .08 | .01 |
| Liver | 50.6 | 6.25 | 43.9 | 6.18 | 26.4 | 2.54 | 10.7 | 1.01 |
| Kidney | 6.25 | 2.80 | 2.46 | 1.41 | .97 | .36 | .79 | .31 |
| Adrenals | .02 | - | .01 | - | .01 | - | .01 | - |
| Thyroid | .01 | - | .01 | - | .01 | - | .01 | - |
| Lymph Gl. | - | .25 | - | .16 | - | .06 | - | .16 |
| Pancreas | .05 | .10 | .05 | .11 | .02 | .02 | .02 | .04 |
| Brain | .01 | .01 | .01 | .01 | .01 | .01 | .02 | .01 |
| Fat | - | .02 | - | .01 | - | .01 | - | .02 |
| Stomach | .50 | .50 | .53 | .25 | .15 | .05 | .14 | .06 |
| Sm. Int. | .94 | .12 | .87 | .12 | .43 | .04 | .27 | .02 |
| Lg. Int. | 1.58 | .31 | 1.40 | .22 | 1.28 | .14 | .33 | .04 |
| Skeleton | 27.0 | 1.53 | 31.3 | 2.15 | 24.2 | 1.07 | 27.5 | 1.20 |
| Muscle | 3.33 | .03 | 2.18 | .03 | 1.37 | .01 | 2.50 | .02 |
| Skin | 3.17 | .10 | 1.47 | .06 | .91 | .02 | 1.38 | .03 |
| Eyes | .01 | .05 | .01 | .05 | .01 | .04 | .01 | .05 |
| Pituitary | .01 | - | .01 | - | .01 | - | .01 | - |
| Gonads | .14 | .04 | .10 | .04 | .06 | .02 | .08 | .02 |
| Urine | 3.62 | - | 8.57 | - | 8.14 | - | 8.28 | - |
| Feces | 1.33 | - | 6.52 | - | 35.7 | - | 47.4 | - |

TABLE II

DEPOSITION OF CARRIER-FREE $Nd^{147}$ COMPLEXED WITH 2 MG. CITRATE IN RATS FOLLOWING INTRAMUSCULAR ADMINISTRATION. VALUES GIVEN AS PERCENT OF ABSORBED DOSE CORRECTED FOR RECOVERY.

| Time After Injection | 1 Day | | 4 Days | | 16 Days | | 32 Days | |
|---|---|---|---|---|---|---|---|---|
| | % per organ | % per gram | % per organ | % per gram | % per organ | % per gram | % per organ | % per gram |
| Heart | .17 | .16 | .13 | .09 | .03 | .03 | .03 | .03 |
| Lungs | .37 | .17 | .23 | .10 | .10 | .05 | .08 | .04 |
| Spleen | .15 | .16 | .11 | .09 | .09 | .09 | .10 | .10 |
| Blood | .08 | .01 | .03 | .01 | .01 | .01 | .05 | .01 |
| Liver | 42.4 | 4.19 | 34.1 | 3.33 | 24.9 | 2.37 | 5.36 | .58 |
| Kidney | 5.16 | 2.25 | 3.42 | 1.31 | 1.02 | .62 | .36 | .18 |
| Adrenals | .01 | - | .01 | - | .01 | - | .01 | - |
| Thyroid | .01 | - | .01 | - | .01 | - | .01 | - |
| Lymph Gl. | - | .16 | - | .22 | - | .10 | - | .08 |
| Pancreas | .04 | .06 | .03 | .05 | .01 | .02 | .02 | .03 |
| Brain | .01 | .01 | .01 | .01 | .01 | .01 | .01 | .01 |
| Fat | - | .01 | - | .01 | - | .01 | - | .02 |
| Stomach | .47 | .24 | .48 | .21 | .13 | .10 | .12 | .03 |
| Sm. Int. | .89 | .10 | .89 | .10 | .88 | .09 | .19 | .02 |
| Lg. Int. | 2.07 | .40 | 2.30 | .38 | 1.87 | .20 | .18 | .02 |
| Skeleton | 32.9 | 2.08 | 33.9 | 2.02 | 24.4 | 1.42 | 22.3 | 1.22 |
| Muscle | 3.22 | .03 | 2.58 | .02 | 1.03 | .01 | 1.84 | .02 |
| Skin | 2.70 | .10 | 2.39 | .06 | .99 | .05 | .60 | .02 |
| Eyes | .01 | .04 | .01 | .07 | .01 | .03 | .02 | .05 |
| Pituitary | .01 | - | .01 | - | .01 | - | .01 | - |
| Gonads | .07 | .02 | .09 | .02 | .03 | .13 | .02 | .07 |
| Urine | 9.24 | - | 9.00 | - | 8.96 | - | 10.9 | - |
| Feces | .09 | - | 10.3 | - | 35.5 | - | 57.8 | - |

TABLE III

THE DEPOSITION OF $Nd^{147}$ COMPLEXED WITH 2 MG. OXALATE FOLLOWING INTRAMUSCULAR ADMINISTRATION. VALUES ARE GIVEN AS PERCENT OF ABSORBED DOSE CORRECTED FOR RECOVERY.

Time After Injection

| | 1 Day | | 4 Days | | 16 Days | | 32 Days | |
|---|---|---|---|---|---|---|---|---|
| | % per organ | % per gram | % per organ | % per gram | % per organ | % per gram | % per organ | % per gram |
| Heart | .13 | .15 | .12 | .11 | .08 | .08 | .07 | .07 |
| Lungs | .39 | .18 | .21 | .13 | .51 | .18 | .18 | .10 |
| Spleen | .17 | .19 | .17 | .22 | .10 | .17 | .34 | .35 |
| Blood | .54 | .04 | .17 | .01 | .06 | <.01 | .23 | .02 |
| Liver | 57.5 | 7.80 | 42.4 | 4.51 | 16.7 | 1.47 | 7.14 | .81 |
| Kidney | 3.93 | 2.12 | 4.09 | 1.65 | 2.48 | .95 | .90 | .43 |
| Adrenals | .01 | - | <.01 | - | <.01 | - | <.02 | - |
| Thyroid | <.01 | - | <.01 | - | .01 | - | <.01 | - |
| Lymph Gl. | - | .26 | - | .16 | - | .10 | - | .09 |
| Pancreas | .03 | .06 | .04 | .05 | .04 | .04 | .02 | .03 |
| Brain | .01 | <.01 | .01 | <.01 | .01 | <.01 | .02 | .02 |
| Fat | - | .01 | - | <.01 | - | .01 | - | <.02 |
| Stomach | .34 | .31 | .74 | .26 | .38 | .08 | .24 | .10 |
| Sm. Int. | 2.43 | .30 | 1.03 | .09 | .64 | .06 | .37 | .04 |
| Lg. Int. | 1.59 | .37 | 1.75 | .24 | 2.00 | .13 | .65 | .11 |
| Skeleton | 24.3 | 1.44 | 27.1 | 1.36 | 29.5 | 1.42 | 36.1 | 1.87 |
| Muscle | 4.66 | .05 | 2.05 | .02 | 6.76 | .06 | 1.45 | <.02 |
| Skin | 1.67 | .06 | 1.80 | .04 | 1.39 | .03 | .73 | .02 |
| Eyes | .02 | .09 | .02 | .06 | .02 | .06 | <.02 | .04 |
| Pituitary | <.01 | - | <.01 | - | <.01 | - | .04 | - |
| Gonads | .01 | .08 | .10 | .03 | .09 | .03 | .07 | .02 |
| Urine | 1.31 | - | 13.6 | - | 12.5 | - | 11.4 | - |
| Feces | .89 | - | 4.51 | - | 26.7 | - | 40.0 | - |

administered remained at the injection site 1, 4, 16 and 32 days after injection.

The results obtained from the administration by intramuscular injection of $Nd^{147}$ to which has been added 1/2 mgm. of stable neodymium are considerably different from what was observed with parenteral administration of carrier-free neodymium as $NdCl_3$ and carrier-free neodymium in the presence of citrate ion and oxalate ion. The major variations noted in the studies in which stable neodymium was added to the $Nd^{147}$ included a significant reduction at the one day interval of Nd in the liver, a very large amount present in the large intestines, probably in the fecal material, a reduced uptake from the skeleton, and a very high level of urinary and fecal excretion, Table IV. Moreover, the absolute values as well as the ratio of urinary and fecal excretion varies at the four time intervals. It is difficult to explain these results, particularly in view of the fact that they differ greatly from the data observed when comparable studies were done using 1/2 mgm. of stable praseodymium as a carrier with $Nd^{147}$. The experiment in which stable praseodymium is added resembles more closely the observations obtained with carrier-free $NdCl_3$, Table V. There is no logical explanation for this apparent fact and it must be stated that in light of the small fraction absorbed from the injection site, errors might have arisen from radioactive contamination, etc. It is certain that these two experiments in which stable Nd and stable Pr are added to the $Nd^{147}$ should be repeated to see if this effect is real. Precaution was taken to avoid possibility of error and the data is presented for its interest.

Three rats received carrier-free $Nd^{147}$ by stomach tube and the animals were sacrificed 8 days afterwards. No measurable radioactivity was found in any of the tissues and organs examined, all of the administered $Nd^{147}$ being recovered in the excreta, Table VI. The presence of this radio-element in the urine is presumed due to contamination from the feces. In view of the fact that the content of $Nd^{147}$ in the liver at 8 days was estimated to be approximately 20 percent of the administered dose and that as little as .01 percent of the administered $Nd^{147}$ could be detected if it were present in the liver, it can be estimated that the absorption from the digestive tract is less than .05 percent of the amount given. It is unlikely that Nd would follow a significantly different metabolic pathway if absorbed from the digestive tract than this element follows when given either by intramuscular or intravenous injection. Moreover, all the other tissues and organs examined contained no measurable amounts of this radio-element. An experiment done with three animals involving intravenous administration of carrier-free $Nd^{147}$ gave results consistent with the intramuscular studies allowing for the fact that these animals were sacrificed after two days instead of the one day interval as was the case in the intramuscular series, Table VII.

The purity of the $Nd^{147}$ was established by taking a fraction of the sample sent from Oak Ridge and searching for the presence of other rare earth activities by means of an ion exchange resin column. An impurity was observed present and the energy of its beta activity together with the fact that Nd did not decay measurably over a period of many weeks indicated it to be $Pr^{147}$. Moreover, the position of the activity of the contaminant with relation to that of $Nd^{147}$ also indicated the contaminant to be Pr. The quantity present at the time the ashed samples from the one day experiments were being assayed was 5 percent. The time intervals for the assay at the 4, 16 and 32 day experiments were such that the theoretical amount of contamination was 7 percent, 12 percent, and 40 percent respectively. However, most of the $Pr^{147}$ activity was cut out by means of a thin aluminum filter and as a result, the measurements of the effective contamination of the samples as they were assayed can be considered negligible.

TABLE IV

THE DEPOSITION OF $Nd^{147}$ IN THE RAT FOLLOWING INJECTION OF 1/2 mg CARRIER Nd USING RADIONEODYMIUM AS A TRACER. VALUES CORRECTED FOR RECOVERY AND GIVEN AS PERCENT OF THE ABSORBED DOSE

Time After Injection

| | 1 Day | | 4 Days | | 16 Days | | 32 Days | |
|---|---|---|---|---|---|---|---|---|
| | % per organ | % per gram | % per organ | % per gram | % per organ | % per gram | % per organ | % per gram |
| Heart | .02 | <.01 | .03 | .02 | <.08 | .10 | .34 | .42 |
| Lungs | .11 | .04 | .09 | .03 | .18 | .08 | .38 | .17 |
| Spleen | .42 | .35 | .53 | .36 | .23 | .23 | .88 | 1.13 |
| Blood | .12 | <.01 | .03 | <.01 | .50 | .03 | .67 | .08 |
| Liver | 14.1 | 1.16 | 18.6 | 1.80 | 4.25 | .45 | 10.4 | 1.34 |
| Kidney | .43 | .15 | .78 | .29 | .28 | .13 | .55 | .29 |
| Adrenals | <.02 | - | <.03 | - | <.08 | - | <.30 | - |
| Thyroid | <.02 | - | <.03 | - | <.08 | - | <.30 | - |
| Lymph Gl. | - | .02 | - | <.01 | - | .38 | - | .92 |
| Pancreas | <.02 | <.02 | <.03 | <.03 | <.08 | .10 | <.30 | .50 |
| Brain | <.02 | <.01 | <.03 | <.01 | <.08 | .05 | <.30 | .17 |
| Fat | - | <.01 | - | <.01 | - | .15 | - | .46 |
| Stomach | .11 | .06 | .12 | .06 | .13 | .05 | .35 | .17 |
| Sm. Int. | .68 | .07 | .34 | .04 | .25 | .03 | .55 | .04 |
| Lg. Int. | 20.8 | 3.86 | 2.42 | .32 | .60 | .08 | .55 | .08 |
| Skeleton | 3.30 | .16 | 7.98 | .35 | 6.33 | .33 | 11.1 | .75 |
| Muscle | .57 | <.01 | .87 | <.01 | 2.00 | <.03 | 5.24 | .08 |
| Skin | .94 | .02 | 1.58 | .03 | .58 | <.03 | 1.51 | .04 |
| Eyes | <.02 | .01 | <.03 | <.01 | <.08 | .25 | <.30 | .84 |
| Pituitary | <.02 | - | <.03 | - | .15 | - | .38 | - |
| Gonads | .02 | <.01 | <.03 | <.01 | .10 | .03 | <.30 | 1.05 |
| Urine | 36.7 | - | 7.48 | - | 47.3 | - | 15.9 | - |
| Feces | 21.7 | - | 59.2 | - | 36.8 | - | 49.4 | - |

TABLE V

THE DEPOSITION OF $Nd^{147}$ IN THE RAT FOLLOWING INJECTION OF 1/2 mg. CARRIER Pr USING RADIO Nd AS A TRACER. VALUES CORRECTED FOR RECOVERY AND GIVEN AS PERCENT OF THE ABSORBED DOSE

| | Time After Injection | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 Day | | 4 Days | | 16 Days | | 32 Days | |
| Tissue | % per organ | % per gram | % per organ | % per gram | % per organ | % per gram | % per organ | % per gram |
| Heart | .08 | .10 | .05 | .05 | <.30 | .30 | 1.33 | 1.60 |
| Lungs | .72 | .30 | .14 | .07 | .34 | .25 | .40 | .22 |
| Spleen | 1.65 | 1.78 | .46 | .41 | .51 | .93 | .67 | 1.24 |
| Blood | .23 | .02 | .14 | <.01 | 1.26 | .13 | 2.04 | .18 |
| Liver | 51.8 | 6.52 | 31.9 | 3.05 | 8.25 | 1.18 | 6.36 | .71 |
| Kidney | 1.08 | .59 | .98 | .43 | .72 | .46 | .44 | .22 |
| Adrenals | .11 | - | <.05 | - | <.30 | - | <.36 | - |
| Thyroid | <.03 | - | <.05 | - | .42 | - | <.36 | - |
| Lymph Gl. | - | 9.19 | - | 3.46 | - | 2.86 | - | 1.47 |
| Pancreas | <.03 | .03 | <.05 | .02 | <.30 | .38 | <.36 | .80 |
| Brain | <.03 | <.01 | <.05 | <.01 | <.30 | .17 | <.36 | .22 |
| Fat | - | .01 | - | <.01 | - | .17 | - | .98 |
| Stomach | .10 | .07 | .11 | .05 | .34 | .21 | .44 | .13 |
| Sm. Int. | .47 | .06 | .40 | .04 | .46 | .04 | .58 | .04 |
| Lg. Int. | 3.79 | .66 | .73 | .13 | 1.09 | .13 | .58 | .04 |
| Skeleton | 13.1 | .76 | 12.1 | .73 | 37.2 | 2.40 | 15.1 | .76 |
| Muscle | 5.67 | .06 | 5.19 | .05 | 11.4 | .17 | 3.78 | .04 |
| Skin | 10.5 | .35 | 7.54 | .19 | 3.45 | .17 | 2.71 | .13 |
| Eyes | <.03 | .04 | <.05 | <.01 | <.30 | .93 | <.36 | .84 |
| Pituitary | .03 | - | <.05 | - | <.30 | - | <.36 | - |
| Gonads | .04 | .25 | <.05 | .02 | <.30 | 1.81 | <.36 | 2.80 |
| Urine | 2.20 | - | 21.6 | - | 10.8 | - | 22.6 | - |
| Feces | 8.40 | - | 18.6 | - | 21.9 | - | 42.3 | - |

TABLE VI

DEPOSITION OF $Nd^{147}$ IN RATS AFTER INTRAVENOUS ADMINISTRATION. VALUES GIVEN IN PERCENT OF ADMINISTERED DOSE CORRECTED FOR RECOVERY

Time After Injection
2 Days

| | % per organ | % per gram |
|---|---|---|
| Heart | .10 | .14 |
| Lungs | .72 | .30 |
| Spleen | .48 | .53 |
| Blood | .05 | <.01 |
| Liver | 46.6 | 6.88 |
| Kidney | 2.10 | 1.33 |
| Adrenals | <.01 | - |
| Thyroid | .02 | - |
| Lymph Gl. | - | .26 |
| Pancreas | .02 | .06 |
| Brain | <.01 | <.01 |
| Fat | - | .01 |
| Stomach | .40 | .21 |
| Sm. Int. | .84 | .11 |
| Lg. Int. | .92 | .19 |
| Skeleton | 33.5 | 1.96 |
| Muscle | 3.67 | .05 |
| Balance | 1.59 | - |
| Skin | 1.58 | .08 |
| Eyes | <.01 | .04 |
| Pituitary | <.01 | - |
| Gonads | .07 | .03 |
| Urine | 5.29 | - |
| Feces | 2.00 | - |

TABLE VII

UPTAKE OF $Nd^{147}$ FOLLOWING ORAL ADMINISTRATION TO RATS. VALUES CORRECTED FOR RECOVERY AND GIVEN AS PERCENT OF DOSE. ANIMALS SACRIFICED 8 DAYS AFTER ADMINISTRATION.

| | % per organ | % per gram |
|---|---|---|
| Heart | <.01 | <.01 |
| Lungs | <.01 | <.01 |
| Spleen | <.01 | <.01 |
| Blood | <.01 | <.01 |
| Liver | <.01 | <.01 |
| Kidney | <.01 | <.01 |
| Adrenals | <.01 | - |
| Thyroid | <.01 | - |
| Lymph Gl. | - | <.01 |
| Pancreas | <.01 | <.01 |
| Brain | <.01 | <.01 |
| Fat | - | <.01 |
| Stomach | <.01 | <.01 |
| Sm. Int. | <.01 | <.01 |
| Lg. Int. | <.01 | <.01 |
| Skeleton | <.01 | <.01 |
| Muscle | <.01 | <.01 |
| Balance | <.01 | - |
| Skin | <.01 | <.01 |
| Eyes | <.01 | <.01 |
| Pituitary | <.01 | - |
| Gonads | <.01 | <.01 |
| Urine | 4.35 | - |
| Feces | 95.6 | - |

Radio Tin. Studies with $Sn^{113}$ have been completed. The fate of tin at time intervals other than 64 days after intramuscular administration and 16 day oral administration has been presented in previous reports. The deposition of tin in the tissues of the rat 64 days after intramuscular administration is shown in Table VIII. The major part of the activity remaining in the animal was found in the skeleton. Measurable amounts of tin were deposited in the liver, kidney, muscle, and skin. Over 70 percent of the radio-tin administered was excreted in the urine and feces. The tin was complexed with citrate when administered and was almost entirely absorbed from the injection site (97 percent).

Oral Administration of $Sn^{113}$. Carrier-free tin is not absorbed to any great extent from the gastrointestinal tract following oral administration. 0.26 percent of the dose was recovered from the skeleton, and .04 percent in muscle 16 days following administration.

Radio-Gallium ($Ga^{67}$). The fate of radio-gallium has been studied in rats. The major isotope used in these studies was $Ga^{67}$ with an 8 hour half-life.

Distribution was studied 1 and 4 days following intramuscular administration. 27 and 23 percent of the gallium administered remained at the injection site. Following absorption, the major organ of deposition was the skeleton. However, the data suggest that this element is not retained for a long period of time since the whole skeleton value dropped from 39 percent at one day after administration to 27 percent four days after. The soft tissues of the body showed measurable uptakes. Gallium is excreted equally in feces and urine. The data are summarized in Table IX.

When radio-gallium is complexed with citrate, absorption is enhanced from the injection site. Only 4 to 5 percent of the $Ga^{67}$ administered remained at the injection site following citrate complexing.

TABLE VIII

DEPOSITION OF CARRIER-FREE RADIO TIN COMPLEXED WITH CITRATE 64 DAYS AFTER INTRAMUSCULAR ADMINISTRATION. VALUES CORRECTED FOR RECOVERY AND EXPRESSED AS PERCENT OF DOSE.

| | Corrected Average | |
|---|---|---|
| | % per organ | % per gram |
| Heart | .01 | <.01 |
| Lungs | .10 | .04 |
| Spleen | .04 | .05 |
| Blood | .03 | <.01 |
| Liver | .40 | .03 |
| Kidney | .24 | .10 |
| Adrenals | <.01 | - |
| Thyroid | <.01 | - |
| Lymph Gl. | - | .04 |
| Pancreas | .02 | .02 |
| Brain | <.01 | <.01 |
| Fat | - | <.01 |
| Stomach | .04 | .02 |
| Sm. Int. | .11 | .01 |
| Lg. Int. | .08 | <.01 |
| Skeleton | 20.0 | .91 |
| Muscle | 1.89 | .02 |
| Skin | 1.32 | .03 |
| Eyes | <.01 | .03 |
| Pituitary | <.01 | - |
| Gonads | .03 | .01 |
| Urine | 54.9 | - |
| Feces | 20.8 | - |
| | 100.0 | |

TABLE IX

DEPOSITION OF RADIO GALLIUM IN THE RAT FOLLOWING INTRAMUSCULAR ADMINISTRATION. VALUES GIVEN AS PERCENT OF DOSE CORRECTED FOR RECOVERY.

| Time After Injection | 1 Day | | 4 Days | |
|---|---|---|---|---|
| | % per organ | % per gram | % per organ | % per gram |
| Heart | .15 | .19 | .09 | .14 |
| Lungs | .42 | .25 | .41 | .23 |
| Spleen | .44 | .46 | .93 | 1.32 |
| Blood | 2.17 | .21 | .61 | .08 |
| Liver | 11.4 | 1.35 | 8.77 | 1.53 |
| Kidney | 1.62 | 1.07 | 1.12 | .84 |
| Adrenals | .06 | - | .03 | - |
| Thyroid | <.02 | - | <.01 | - |
| Lymph Gl. | - | .46 | - | .67 |
| Pancreas | .06 | .19 | .09 | .23 |
| Brain | .08 | .06 | .06 | .05 |
| Fat | - | .11 | - | .03 |
| Stomach | .86 | .42 | 1.15 | .73 |
| Sm. Int. | 4.91 | .51 | 1.68 | .26 |
| Lg. Int. | 1.77 | .29 | 1.16 | .20 |
| Skeleton | 39.2 | 2.26 | 27.1 | 1.70 |
| Muscle | 7.57 | .10 | 3.04 | .05 |
| Skin | 3.84 | .17 | 2.00 | .11 |
| Pituitary | <.02 | - | <.01 | - |
| Gonads | .10 | .38 | .06 | .40 |
| Urine | 11.4 | - | 24.6 | - |
| Feces | 14.0 | - | 27.1 | - |

## Decontamination and Bone Metabolism Studies

### Effect of Carrier Zirconium on Blood Curves, Tissue Uptake and Urinary Excretion of Injected $Zr^{95}$ - $Cb^{95}$.

Carrier zirconium (as citrate) appears to be one of the most effective means of increasing the acute elimination of plutonium, yttrium, and other metallic elements involved in fission. The following experiment was undertaken to study the effects of carrier zirconium on the blood curve, tissue distribution and excretion of injected $Zr^{95}$, $Cb^{95}$ during the critical first hour following administration.

$Zr^{95}$ decays with a half-life of 65 days to $Cb^{95}$, which in turn has a 35 day half-life. Because of the similarity of half-life, and of radiation (beta and gamma) from these two isotopes, it is difficult to distinguish the parent and daughter by their decay characteristics. Since it is also difficult to separate them chemically, the two isotopes were administered and measured as a unit. By measuring the activity of the samples four months hence and comparing the decay with that of the standard mixture, it is hoped to determine if there is any difference in the distribution of these two isotopes in the tissues studied.

Experimental Procedure. Adult female rats (weight 250-350 grams) maintained on stock diet were injected simultaneously with 5 microcuries of the $Zr^{95}$-$Cb^{95}$ (65 percent Zr, 35 percent Cb) mixture and 40 mg. of carrier zirconium as citrate. Both were given intraperitoneally. The pH of the solution was 6, and the total volume administered per rat was 1.6 cc. The rats were sacrificed in groups of 3 at 5, 10, 15, 30 and 60 minutes following injection. Liver, kidneys, spleen, one femur, and a blood sample were removed and analyzed for radioactivity. Feces were collected from the chamber and by direct removal from the large intestine; urine was obtained from the chamber and by washing out the urinary bladder. The results of the assays for radioactive material are given in Table X; the curves derived from this data are shown in Figure 1.

Observations. Active absorption from the peritoneal cavity is indicated by the significant blood level of $Zr^{95}$-$Cb^{95}$ within 5 minutes after injection, reaching a peak value around 15 minutes. However, a large amount of the dose is still unabsorbed in the peritoneal cavity at the end of the first hour. There is a continuous uptake of the $Zr^{95}$-$Cb^{95}$ mixture by bone throughout the period, and a very significant excretion in urine. At the end of the hour, over five times as much as has been excreted in urine has been deposited in bone. This conforms with previous experience in which carrier zirconium citrate increased urinary excretion and decreased bone deposition. It is interesting that such a marked effect should be obtained so early. The renal clearance of the $Zr^{95}$-$Cb^{95}$ appears to be fairly constant over this interval (1/2 cc. per minute).

### Bone Metabolism Studies with Radiostrontium.

Radiostrontium is one of the most hazardous products of fission from the point of contamination. Since it closely resembles calcium in its biological behavior, it also provides a valuable means for studying fundamental bone metabolism. The following experiments are part of a general study of bone metabolism with radiostrontium, considered from both these aspects. It is soon to be supplemented by work with radiocalcium.

TABLE X

EFFECT OF CARRIER ZIRCONIUM ON THE TISSUE DISTRIBUTION AND EXCRETION OF $Zr^{95}$ - $Cb^{95}$

| | Percent of the Administered Dose of $Zr^{95}$ - $Cb^{95}$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Time | Blood* | Femur | Skeleton** | Liver | Kidney | Spleen | Feces | Urine |
| 5 Minutes | 5.4 | 0.02 | 0.35 | 2.2 | 1.0 | 0.3 | 0.6 | 0.4 |
| 10 Minutes | 4.8 | 0.006 | 1.1 | 3.5 | 1.0 | 0.3 | 0.8 | 1.4 |
| 15 Minutes | 9.2 | 0.09 | 1.6 | 2.1 | 1.1 | 0.4 | 0.3 | 3.0 |
| 30 Minutes | 5.1 | 0.12 | 2.0 | 2.4 | 1.5 | 0.2 | 1.7 | 7.2 |
| 60 Minutes | 3.9 | 0.16 | 2.6 | 1.6 | 2.1 | 0.1 | 0.1 | 13.9 |

* The value for total blood was calculated on an estimated total blood mass of 6 percent of the body weight.

** The value for total skeleton was estimated from the femur value in relation to the total skeleton mass as given in "The Rat" by Donaldson.

Effect of Severe Rickets on the Deposition of Radiostrontium in Bone. Severe rickets is characterized by interference with calcification of bone and a marked reduction in the retention of radiostrontium in the skeleton. However, certain experiments reported earlier have indicated that the initial uptake of radiostrontium by rachitic bone (during the first hour) may be quite rapid. In the following experiment, severe rickets was produced in rats by restricting them to a diet very low in phosphorus from the time they were weaned (at 21 days of age). After 6 weeks on the experimental diet, the rats were injected with 5 microcuries of carrier-free $Sr^{89,90}$ and placed in individual metabolism cages. Five animals were sacrificed after 1 hour, while another 5 were sacrificed after 72 hours (3 days). A control group of rats weaned at the same time, and fed the experimental diet supplemented with phosphate, were similarly injected. Femur, tibia, incisor teeth, molars, and urine and feces were analyzed for radiostrontium. Costochondral junctions (which showed a marked enlargement in the rachitic animals) were taken for radioautographic study to demonstrate the actual site of radiostrontium deposition in this area, particularly with respect to the uncalcified osteoid present, and the calcified cartilage.

Preliminary results showed that in both rachitic and control groups a large part of the radiostrontium (over 75 percent) was deposited in the skeleton at 1 hour. In the control animals, this radiostrontium appeared to remain fixed in the bone, for the amount in the skeleton at 72 hours was equal to that found at 1 hour. In the rachitic animals, the radiostrontium originally fixed in the bone was largely eliminated within the first 72 hours, and the quantity remaining in the skeleton at this time was less than 20 percent. Loss of radiostrontium was almost entirely by way of the urine. Indeed, even in the first hour, the urinary excretion of radiostrontium was over 5 times as great in the rachitic rats as it was in the controls.

The lability of the skeletal deposits in the rachitic animals, and the high level of urinary excretion suggests that this procedure may be of value in decontamination of radiostrontium. While the osteoid matrix, so prominent in the rachitic bone, might be considered as a site of labile fixation of radiostrontium, radioautographs of the costochondral junction of animals sacrificed at 1 hour show the radioactive material only in areas of bone salt with none in the uncalcified matrix. Further work is planned to study the mechanism involved.

Deposition of Radiostrontium in rats Thyroidectomized at Birth and the Effect of Thyroxin and Growth Hormone. Various endocrine factors involved in bone metabolism are being studied to determine their possible value in the problem of decontamination. In the following experiment, the behavior of rats thyroidectomized at birth was investigated.

The rats were weaned at 3 weeks, and sacrificed after 60-70 days. Thyroxin treated rats received 2.0 micrograms of thyroxine daily for the last month; during the same period growth hormone treated animals received 0.5 mg. per day for 10 days; 0.75 mg. per day for 10 days, and 1.0 mg. per day for the last 10 days. A group of normal rats similarly maintained on stock diet served as controls. Forty-eight hours before the animals were sacrificed they were given 10 microcuries of carrier-free $Sr^{89,90}$ in isotonic saline. The uptake of this radiostrontium in femur and tibia was determined. The results are given in Table XI.

TABLE XI

UPTAKE OF RADIOSTRONTIUM BY BONE IN THYROIDECTOMIZED RATS AND EFFECT OF TREATMENT

| Group | Percent of Administered Dose of $Sr^{89,90}$ | |
|---|---|---|
| | Femur | Tibia |
| 1. Normal controls | 2.9 ± 0.5 | 2.2 ± 0.3 |
| 2. Thyroidectomized with Thyroxin and GH | 3.3 ± 0.5 | 2.2 ± 0.2 |
| 3. Thyroidectomized (no treatment) | 1.7 ± 0.2 | 1.2 ± 0.2 |
| 4. Thyroidectomized with Growth Hormone | 2.7 ± 0.1 | 1.7 ± 0.1 |
| 5. Thyroidectomized with Thyroxin | 2.7 ± 0.9 | 2.0 ± 0.1 |

(Values given are averages for 3 - 5 animals, with the standard deviation)

Observations. Thyroidectomy at birth, producing rat cretinism, inhibits the deposition of radiostrontium in the skeleton. Growth hormone and thyroxin, either alone or together, increase the skeletal uptake of $Sr^{89,90}$ to a level which may even exceed that of the normal controls.

Other Work in Progress.

1. Study of the effect of estrogens on the deposition of radiostrontium in bones of rats and mice.

2. Kinetics studies of uptake of $P^{32}$ (phosphate) by bone.

3. Metabolism of radiofluorine - $F^{18}$.

4. Certain studies of bone metabolism with $Ca^{45}$.

5. Radioautographic studies of deposition of radioactive isotopes and fission products in normal and rachitic bone, in collaboration with D. Heller and C. W. Asling.

6. Studies of bone structure with the electron microscope.

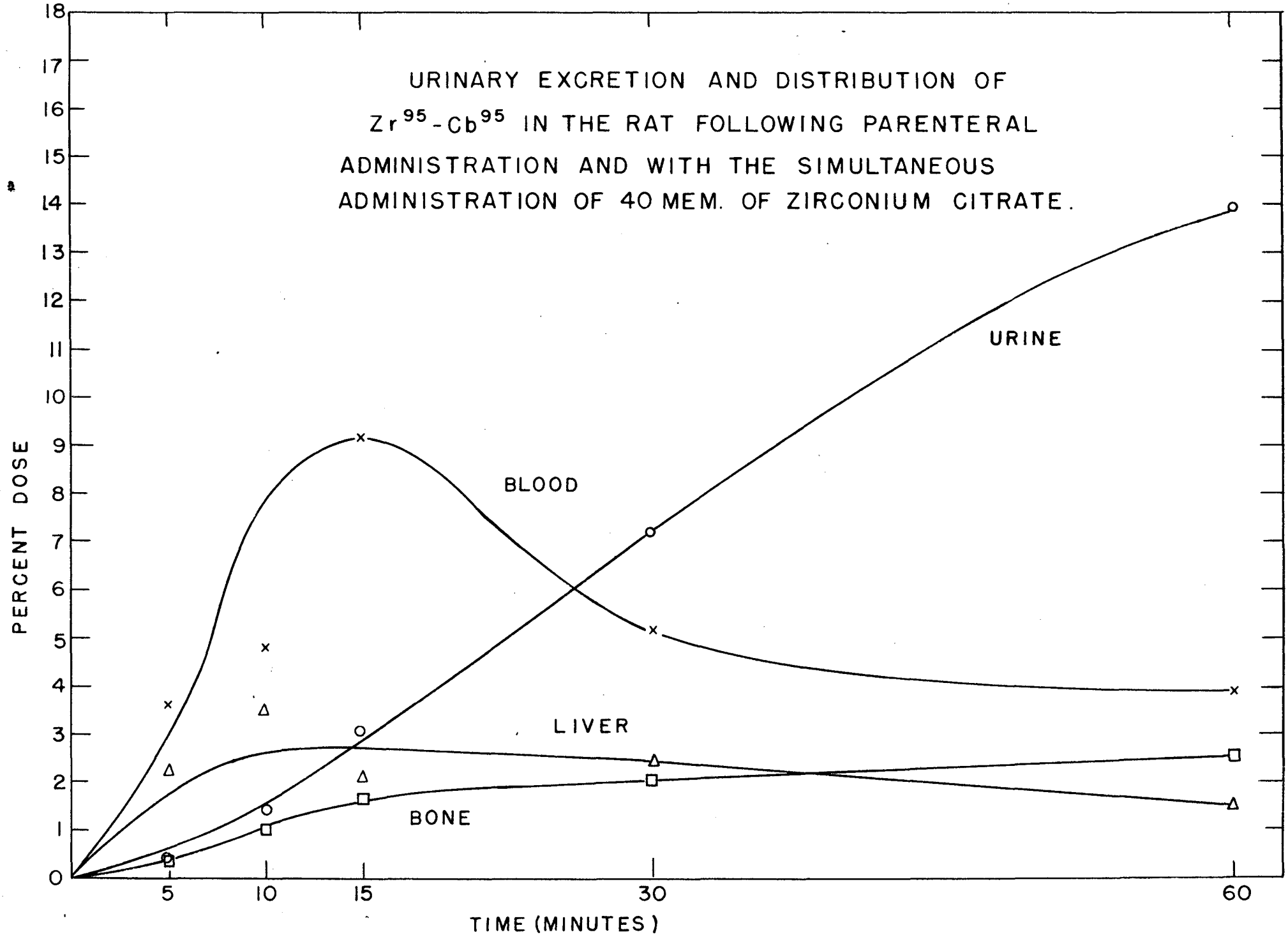
URINARY EXCRETION AND DISTRIBUTION OF
Zr95-Cb95 IN THE RAT FOLLOWING PARENTERAL
ADMINISTRATION AND WITH THE SIMULTANEOUS
ADMINISTRATION OF 40 MEM. OF ZIRCONIUM CITRATE.
URINE
BLOOD
LIVER
BONE
PERCENT DOSE
TIME (MINUTES)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
5
10
15
30
60

FIG. 1

13669-4

## Radiochemistry

A method was developed for separating carrier-free selenium activity made by the reaction $As^{75}(d,2n)Se^{75}$. After dissolving the arsenic in a minimum volume of boiling aqua-regia, excess concentrated HCl was added and the solution diluted to 3N. Ten milligrams of tellurium as tellurious acid was precipitated from this solution with $SO_2$. The activity carried quantitatively. The precipitate washed, re-precipitated and dissolved in a minimum volume of $HNO_3$. Excess HBr was added and the carrier-free $Se^{75}$ was distilled from the $HBr-Br_2$ mixture. The distillate containing the $Se^{75}$ was treated with concentrated $HNO_3$ to remove HBr and evaporated to dryness after the addition of excess HCl and 5 ml. of isotonic saline.

Approximately 3.5 millicuries of $Cu^{64}$ and 15 millicuries of $Ga^{67}$ were isolated carrier-free from deuteron bombarded zinc. The target was dissolved in HCl and the pH adjusted to 5.5 with NaOH. The solution was extracted several times with ether to remove radio-gallium. The aqueous phase was taken nearly to dryness, adjusted to pH 1-1.2 and extracted with .001 percent dithizone in $CCl_4$. The combined dithizone $CCl_4$ extract was washed with 0.1N HCl which re-extracted any traces of Zn. The $Cu^{64}$ was recovered from the dithizone complex by evaporating the $CCl_4$ solution to dryness on NaCl followed by ignition at dull redness for 15 minutes.

Work on astatine was continued. A new target was designed to obtain maximum counting efficiency. The $X/\gamma$ counting ratio was found to be 1/850 with geometry approximating that to be encountered in thyroid studies in humans. Several types of GM tubes have been investigated to obtain maximum counting efficiency for the $At^{211}$ x-radiation. A silver cathode tube filled with atmospheric helium and alcohol gave the most satisfactory plateau. Argon, krypton, and xenon are also being studied.

Several preliminary tracer studies have been made with rats and guinea pigs. Data in Table XII, A, B, C, show the distribution found in rats 18 hours after injection. The results are of qualitative significance only. Assay was made by macerating a sample of tissue in a porcelain counting dish with aqueous KOH. Total dry solids did not exceed 3 $mg/cm^2$. The samples were counted with a nitrogen counter in the proportional region.

A more satisfactory method of assay has been developed which eliminates the mass-absorption errors inherent in the KOH technique. The analysis is based on the observation of Johnson, Leininger, and Segrè that At is carried by a metallic tellurium precipitate from 3N HCl saturated with $SO_2$. After wet washing, the tissues with aqua-regia, quantitative recoveries of added At activity were obtained by precipitation on 3 milligrams of Te with $SO_2$ from 10 ml. of 3N HCl, containing the soluble oxidation products from approximately 2 grams of tissue. After precipitation, the Te was washed with the $SO_2$ saturated 3N HCl, centrifuged and transferred to a porcelain counting dish with distilled water. At tracer studies now in progress are being assayed with this technique. Results for one animal are shown in Column D. The laboriousness of the procedure and the 7.5 hour half-life of $At^{211}$ precludes the isolation and assay of $At^{211}$ from more than 20 to 30 specimens of tissue.

A separate report on the electrical transference method of separating carrier-free activities was prepared. The method is being used routinely in the separation of carrier-free $Cb^{95}$ from Cb-Zr mixtures.

TABLE XII

THE DISTRIBUTION OF $At^{211}$ IN THE RAT 18 HOURS FOLLOWING INTRA-MUSCULAR INJECTION INTO THE LEFT LEG.

| | A | | B | | C | | D | |
|---|---|---|---|---|---|---|---|---|
| Tissue | % per organ | % per gram | % per organ | % per gram | % per organ | % per gram | % per organ | % per gram |
| Heart | .11 | .12 | .17 | .18 | .14 | .13 | .06 | .05 |
| Lung | .65 | .55 | 1.30 | .90 | 1.65 | 1.09 | 4.57 | 2.68 |
| Spleen | 1.18 | .58 | 1.42 | 1.46 | .89 | 1.13 | 3.08 | 5.27 |
| Blood | 1.97 | .15 | 2.62 | .20 | 1.31 | .10 | | |
| Liver | 1.61 | .13 | 3.35 | .26 | 1.69 | .15 | 5.15 | .40 |
| Kidney | .25 | .10 | .91 | .37 | .61 | .29 | 1.42 | .61 |
| Adrenal | .01 | - | .02 | - | <.01 | - | 0.08 | 1.12 |
| Thyroid | 1.07 | 53.9 | 2.06 | 68.5 | .31 | 10.1 | 4.80 | 160. |
| Lymph Gland | - | .29 | - | .26 | - | .07 | .01 | - |
| Pancreas | - | .44 | - | .36 | - | .30 | .95 | 1.22 |
| Brain | .02 | .01 | .08 | .05 | .04 | .03 | .01 | - |
| Salivary Gland | - | .23 | - | .19 | - | .22 | - | - |
| Stomach | 7.17 | 5.56 | 7.52 | 5.49 | 7.46 | 4.60 | 2.18 | .72 |
| Stomach content | 8.45 | - | 25.9 | - | 1.21 | - | | |
| Sm. Int. | 4.92 | .80 | 5.36 | .67 | 3.74 | .62 | 2.42 | .17 |
| Sm. Int. content | 2.34 | - | .86 | - | 2.16 | - | | |
| Lg. Int. | .47 | .22 | .96 | .60 | 1.61 | .53 | 7.82 | .80 |
| Lg. Int. content | .76 | - | .98 | - | .89 | - | | |
| Left Leg | 15.7 | - | | - | | - | 9.52 | .61 |
| Muscle | 3.74 | .038 | 6.79 | .067 | 2.07 | .021 | .01 | - |
| Skeleton | 5.51 | .18 | 5.51* | .18* | 5.51* | .18 | 2.30 | |
| Balance | 7.08 | - | | - | | - | | |
| Skin | 19.2 | .56 | 21.2 | .56* | 20.7 | .56* | 13.70 | .28 |
| Trachea | .08 | - | .18 | - | - | - | - | - |
| Gonads | 1.00 | .28 | 1.30 | .41 | .71 | .30 | .75 | .27 |
| Urine | 7.10 | - | 7.10 | - | 7.10 | - | - | ** |
| Feces | .57 | - | .57 | - | .57 | - | - | - |

* Values taken from A. Disregarding left leg and balance.

** Urine and Feces not analyzed.

10.7 millicuries of $Cu^{64}$ were obtained from a deuteron bombarded copper target. The final solution had a specific activity of 18.2 millicuries per gram of copper.

385 microcuries of carrier-free As were separated from a deuteron bombarded germanium target using a previously described method.

The ion exchange column was used for identification of Nd, Pr, and Y activities received from Oak Ridge during the past quarter. Dowex 50, 250-500 mesh was used and the activity eluted from the complex by 5 percent citrate, pH 3.03. The Nd was found to have approximately 5 percent of a second activity which by energy and its proximity to Nd from column elution together with long half-life was assumed to be element 61. The Pr and $Y^{91}$ activities proved to be single activities.

In order to prove chemically that the activities were as indicated, carrier was added to each of the above solutions. The peak of the activity was found to be at the peak of the carrier as shown by emission spectrographic analysis.

# II BIOLOGICAL STUDIES OF RADIATION EFFECTS

J. H. Lawrence - in charge

Project 48A-II

## Blood Coagulation in Polycythemia and Leukemia; Relation of Heparin and Platelets; Quantitative Measure of Clot Retraction and Heparin Clotting Time

Robert L. Rosenthal

### Introduction

The occurrence of thrombosis and hemorrhage are two of the most prominent causes of complications and morbidity in disease. This is especially true of polycythemia vera and leukemia. Thrombosis, especially of the cerebral and coronary vessels, represents the most serious threat to life in polycythemia[1,2,3,4]. Curiously enough, hemorrhage also occurs in this disease. It is a frequent symptom in the form of epistaxis and bleeding gums or may be quite severe after any type of surgical operation, even tooth extraction[5,6,7]. In leukemia, hemorrhage has long been a widely recognized finding and therapeutic problem[1,8].

The value of studying the blood coagulation mechanisms in these diseases is readily apparent. However a review of the literature reveals no adequate or extensive study of coagulation in these conditions except for a study on thrombocytopenic purpura in leukemia by Rosenthal[9]. The available information on this subject comes from various clotting tests performed on small numbers of cases in many different clinics. These tests, which usually consist of platelet count, clotting time, bleeding time, presence of clot retraction, prothrombin time and capillary fragility, have been generally admitted to show wide variation from laboratory to laboratory, depending upon the specific details of the method and the subjective attitudes of the person performing the test[10]. Some of these tests, at best, are only qualitative and are of limited value in many circumstances. Many tests used in experimental studies on blood coagulation are either unproven or are too laborious to be adaptable to routine clinical use. Few new tests of clinical value have been introduced.

In his monograph on leukemia, Forkner[11] stated, "Frequently no adequate explanation can be advanced as to the cause of bleeding in leukemia. The problem has been studied inadequately."

It is the purpose of this paper to report the results of a one and one-half year study of blood coagulation on 45 patients with polycythemia vera, 28 patients with leukemia and 16 patients with other diseases. During the course of this study, two new tests of blood coagulation were developed and carefully standardized upon 64 normal subjects. These tests are:

1) Clot retraction rate, a quantitative measure of clot retraction obtained by electric resistance measurements.

2) Heparin clotting time, a modification and simplification of the heparin tolerance test, which measures the effect of added heparin upon the clotting time.

The results indicate that these tests are of great clinical and experimental value.

For convenience, this report is divided into sections as follows:

Part I. Coagulation Tests

A. Clot Retraction Rate
B. Heparin Clotting Time
C. Methods of Other Tests

Part II. Results

A. Plan of Study
B. Leukemia
C. Polycythemia Vera
D. Other Patients
E. Effect of Treatment with Radioactive Isotopes on Coagulation
F. Statistical Analysis of Data

Discussion

Summary

## Part I. Coagulation Tests

### A. Clot Retraction Rate

Review of the Literature. About 30 minutes after a blood clot has formed in a glass tube, the clot is seen to separate from the wall of the tube. This is caused by the twisting and shortening of the fibrin strands of the clot under the influence of intact platelets[12,13,14,15]. As the process continues, the red cells caught in the fibrin network are packed together and the serum is expressed out to the periphery. The relation between clot retraction and platelets was first noted by Hayem[16] who observed deficient clot retraction in cases of thrombocytopenic purpura.

In general, clot retraction has been of little practical value as a test, being used chiefly as an approximate confirmatory test of platelet function. The platelet count, clotting time, bleeding time and capillary fragility tests have received more attention. The reason for this neglected status of clot retraction lies in the lack of adequate methods available for its measurement. Most methods have been largely subjective. They consist of observing whether or not the clot has retracted or the time at which retraction is first noted[9,17,18,19,20,21]. Semi-quantitative measurements have been attempted by removal of the retracted clot at a certain time and obtaining its volume, corrected for hematocrit, by measuring the serum left in the tube[22,23,24,25,26].

Quantitative Measurement. A method* was developed and described by Rosenthal and Tobias[27] by which a quantitative measure of clot retraction, as well as other information, may be obtained by measurement of the electrical resistance of freshly drawn blood, as it clots. Fig. 1 shows a typical electric resistance curve with explanations and illustrations of the changes. The measurement is based upon the retraction of the clot around the platinized platinum electrodes.

The red cells are poor conductors of electricity. As the clot retracts and the serum is displaced to the periphery, the red cell concentration around the electrodes is increased to give corresponding increases in the electric resistance. Fig. 2 shows the "average normal curve" and the range for 90 percent of the subjects based on observations on 52 normal subjects, 35 males and 17 females. It will be noted that the resistance shows the greatest increase during the first hour of measurement. Thereafter, the rate of increase becomes progressively smaller. The range also shows much greater spread after the first hour. Duplicate measurements on aliquot samples of blood show that the greatest accuracy and least amount of error is obtained during the first forty minutes. Theoretically, errors and inconsistencies would result if the retracting clot fails to surround the electrodes completely or if the electrode edges cut through the clot. This is more likely to occur after a period of time when the retracting clot has become smaller. This possibility especially must be considered in pathological conditions where clot retraction is more rapid or where the hematocrit is so low that the clot is too small to surround the electrodes. Experience has shown that these factors are negligible for at least thirty to forty minutes after clot retraction begins. The possible occurrence of this type of error may be obviated by the use of round electrodes.

It has been postulated that the resistance increase is caused by clot retraction. Therefore, the slope, which denotes the rate of increase of the resistance curve, equals the "clot retraction rate". Since the first forty minutes of measurement are least susceptible to error owing to the factors mentioned above, the slope of the resistance curve between 10 and 30 minutes has been used in the calculation of the clot retraction rate as follows:

$$\text{Clot retraction rate in ohm-cm. per minute} = \frac{\text{Specific resistance at 30 minutes} - \text{Specific resistance at 10 minutes}}{\text{Time at 30 minutes} - \text{Time at 10 minutes}}$$

The single value, known as the clot retraction rate, is an expression of the quantitative measurement of clot retraction. For 53 normal subjects, the average value was 7.1 ohm-cm per minute and the 95 percent range of plus and minus two standard deviations was 4.1 to 10.1 (Table I). The distribution of the clot retraction rates in normal subjects and patients is shown in Fig. 3. For the normal subjects, the presence of two peaks can be seen at 6 to 7 and 8 to 9 ohm-cm. per minute. The first range corresponds to the peak for the 17 female subjects, the second to that for the 35 male subjects. However, the difference between the average values for the males and females was not statistically significant. On the basis of 12 duplicate measurements on aliquot samples of blood, the average error of the clot retraction rate was about 10 percent.

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* The method of resistance measurement was revised by Dr. C. W. Tobias, assisted by Mr. Leo Lipetz. Measurements, since June, 1948, have been obtained by comparing the potential across the electrodes to a standard potential. As a constant current is used, the resultant potential across the electrodes is directly related to the resistance. Measurements are recorded automatically by a Speedomax (Leeds and Northrup) every 12 seconds for each sample. Five samples can be measured simultaneously.

TABLE I

VALUES FOR NORMAL SUBJECTS OF TESTS USED IN THIS STUDY

| Test and Units | Average Value | 90 Percent Range | |
|---|---|---|---|
| | | High | Low |
| Heparin clotting time (minutes) | 27.5 | 34.7* | 20.3* |
| Clot retraction rate (ohm-cm. per minute) | 7.1 | 10.1* | 4.1* |
| Clotting time (min.) 100 x 13 mm. tube | 7.5 | 10.5 | 5.0 |
| (1)75 x 10 mm. tube | 6.4 | 9.0 | 4.5 |
| (2)75 x 10 mm. tube | 9.4 | 13.0 | 6.0 |
| Bleeding time (min) | 3.8 | 6.0 | 2.5 |
| Platelet count (per c.mm.) | - | 400,000 | 200,000 |
| Prothrombin time (seconds) 100 % plasma | 16.3 | 18.4 | 14.1 |
| 10 % plasma | 29.4 | 35.2 | 24.3 |
| Hematocrit (per cent) | 46.2 | 51.0 | 40.5 |

* Values represent plus or minus two standard deviations ($\sigma$).

Fig. 4 shows the average curves, whose slopes represent the clot retraction rate, for various groups: normal, leukemia (showing typical curves), and polycythemia. The polycythemia group has been further subdivided into those with platelet counts above and below 400,000. There is a similarity in clot retraction rate or slope of the curve among the normal, leukemic and polycythemic subjects with platelet count below 400,000. However, there are marked differences in the initial resistance value since it is comparable to the hematocrit. On the other hand, there is a markedly higher clot retraction rate in the polycythemia group with platelet counts above 400,000.

Observations. The electric resistance curve shows that clot retraction begins almost as soon as a firm clot has formed. It appears that the rate of clot retraction is greatest for the first 30 minutes after which it progressively decreases. We have observed that clot retraction continues for over 24 hours. Measurements on both recalcified oxalated plasma and blood gave no change in electric resistance, although solid clots formed. Neither showed any external evidence of clot retraction after 24 hours.

Hirschboeck[28] made the interesting observation that clot retraction does not occur in collodion-lined tubes. Apparently, collodion has a strong affinity for fibrin and thus prevents it from separating from the walls of the tube. Fig. 2 shows the electric resistance curves on aliquot samples of blood with both glass and collodion-lined tubes. The curves are identical up to a point close to 30 minutes when the resistance in the collodion-lined tube shows no further rise. This point corresponds to the time at which the clot in the glass tube is seen to separate from the wall. No clot retraction was seen in the collodion-lined tube for over 24 hours. Apparently, normal internal clot retraction occurs in collodion-lined tubes until separation from the wall must occur in order to obtain further retraction and resultant increase in resistance.

The occurrence of clot retraction at its maximum rate within the first 30 minutes adds credence to suggestions that clot retraction plays an important physiological role in the maintenance of hemostasis by pulling together the walls of open blood vessels.[29] No change can be detected by gross examination of the clot in the tube until separation of the clot from the wall begins.

Preliminary experiments revealed that heparin in small concentrations delays the start of clot retraction as well as the formation of a firm clot but does not affect the clot retraction rate.

## B. Heparin Clotting Time

Review of the Literature. The use of heparin in blood coagulation tests was prompted by the observation of Crafoord[30] in 1937 that post-operative patients showed less prolongation of clotting time after the administration of heparin than did normal subjects. De Takats[31] injected a standard amount of heparin and measured its effect on the clotting time of subjects 10 and 20 minutes after injection. He called that a "heparin tolerance test". Waugh and Ruddick[32,33] then modified this test by adding varying amounts of heparin to blood in vitro, obtaining the clotting time and plotting heparin concentration versus clotting time. Numerous variations of the test have since been reported[34-39], and it has been found that in vivo and in vitro tests give similar results[40,41].

By this test, an increased blood coagulability or increased tolerance to heparin was found in various conditions where there is a tendency towards thrombosis[36,37,42,44] and embolism such as after surgery[31,32,33,39] and in myocardial infarctions[43]. This test had been used exclusively for the detection of increased coagulability until Allen et al.[38] reported increased clotting sensitivity to heparin in a few patients with thrombocytopenic purpura.

Despite its potential value, the clinical use of heparin in blood coagulation tests has been greatly under-developed. This can be attributed to the complex and time-consuming nature of present heparin clotting tests which have been inadequately standardized and difficult to interpret. These tests tend to give variable results[45].

It is our purpose to describe a heparin clotting test and its standardization and simplification. The test consists of mixing a single concentration of heparin (.004 mg.) with 1 cc. of freshly drawn blood and determining the clotting time. Since the test does not involve injecting heparin into the subject, the widely used term "heparin tolerance" is both inaccurate and confusing. Therefore, the present test is referred to as the "heparin prolonged clotting time" or " heparin clotting time". Although its performance is easy, a standard technique must be followed in order to obtain accurate and consistent results.

Method of Standardization. The effect of various concentrations of heparin on the clotting time was first established. Preparation of the tubes and heparin solutions was as follows:

Dry glass tubes, 13 by 100 mm. were filled with 1.1 cc. of water carefully measured with a 1cc. and a .1 cc. pipette. In order to obtain an accurate volume, the water must be at the bottom of the tube and adherence to the sides avoided. A noticeable scratch mark was then placed on the tube level with the bottom of the meniscus of the fluid. Before use, tubes were washed, rinsed in cleaning solution and distilled water, and thoroughly dried in an oven.

A series of six different dilutions of heparin* in isotonic (.85%) saline was prepared as listed in Table II, which shows the heparin concentration in milligrams (mg.) per .1 cc of saline. When kept well stoppered in the refrigerator, the heparin solution maintains a constant potency for several months.

For the test, 8 tubes prepared as above, were placed in a test tube rack immersed in a constant temperature water bath set at 37 degrees centigrade. Tubes were numbered 0 to 7. .1 cc. of each heparin solution was placed in the appropriate tube as indicated in Table II. Care was taken to avoid adherence of the solution to the sides of the tube. Tube 0, which contained neither saline nor heparin, and Tube 1, which contained saline without heparin, served as controls and corresponded to the ordinary clotting time.

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* Heparin made by Lederle was used in this study. Most commercial preparations which may be used in this test consist of the purified sodium salt of heparin in solution with 1 cc. containing 10 mg. of heparin.

TABLE II

HEPARIN CONCENTRATIONS AND CLOTTING TIMES FOR 20 NORMAL SUBJECTS

| Tube number | Heparin Concentration in .1 cc. .85% saline | Clotting times for normal subjects | | |
|---|---|---|---|---|
| | | Minimum | Average | Maximum |
| | milligrams | min. | min. | min. |
| 0 | - | 4.5 | 7.0 | 10.0 |
| 1 | .000 | 4.5 | 6.4 | 9.0 |
| 2 | .002 | 13 | 16.4 | 19 |
| 3 | .004 | 22 | 26.7 | 36 |
| 4 | .006 | 29 | 38.9 | 46 |
| 5 | .008 | 42 | 53.5 | 62 |
| 6 | .010 | 52 | 73.9 | 92 |
| 7 | .012 | 86 | 116.1 | 197 |

Blood was obtained with a dry syringe and 20 gauge needle. It is very important to enter the vein immediately and cleanly as any admixture with tissue juice completely distorts the results of the test. Enough blood was taken so that the first and last cc. in the syringe were not used for this test. Time was counted as soon as about half the blood had been drawn into the syringe. With the needle removed from the syringe, blood was placed in each of the prepared tubes by allowing it to run down the side until the bottom of the meniscus of the blood corresponded to the scratch mark level. Thus, 1cc. of blood was placed in each tube. The outcome of the test was found to be very closely related to the amount of blood added. Holding the tube near the eye in good light facilitates this step which requires some practice. If the blood meniscus cannot be seen, as is sometimes the case, the blood level at the side wall of the tube is the guide. This level should come slightly above the scratch mark as it does when the meniscus can be seen. Experience showed that this phase of the test accounted for marked discrepancies until these precautions were exercised. More accurate measurement of the blood, as by the use of a pipette, was found to be unnecessary.

After the tubes were filled, the heparin was mixed with the blood by completely inverting each tube twice, once in each direction. It was found convenient to cover the tube with a piece of plastic material held by the finger. After mixing, a stopper was inserted. Tube 0 was not moved in this step.

The clotting times were determined as follows: The end point was taken as the time of formation of a solid clot that remained in situ on complete inversion of the tube. Since variations in movement alone were found to alter the clotting time, this factor was minimized and made as constant as possible by tilting the tubes just enough to see whether or not the blood was clotted. Tubes 0 and 1 were inspected at half-minute intervals after the three minute mark. When both were clotted, Tube 2 was then inspected at one minute intervals until clotted, at which time Tube 3 was inspected at one minute intervals and so on. No tube was moved until its predecessor had clotted. It was important to follow these details in order to obtain satisfactory and accurate end points, especially for tubes clotting in over 2 hours. The use of recalcified oxalated plasma instead of blood, as reported by Silverman[39], was attempted and abandoned because of the inability to obtain satisfactory or reliable end points.

Observations. The results of this test in 20 normal subjects, 11 males and 9 females, are shown in Table II. It is to be noted that in the last few tubes the increase in heparin concentration had a proportionately greater prolongation effect on the clotting time. A plot of the data on semi-longarithmic paper, with the heparin concentration on the linear scale and clotting time on the log scale, revealed a straight line curve as shown in Figure 5. This suggested a logarithmic relation between heparin concentration and clotting time which has also been reported by Jaques[41] and Best[46]. It was also noted that, although a straight line could be drawn through the clotting times of Tubes 3 (.004 mg. heparin) to 7 (.012 mg.), the clotting times of Tubes 1 and 2 fell below this straight line. This would indicate that heparin did not exert its maximum anticoagulant effect until Tube 3. This is consistent with reports on both the in vivo and in vitro potentiation of the anti-coagulant effect of heparin after it has incubated with the blood for about 10 to 15 minutes[41]. Hence, Tube 2 clotted before the heparin could exert its maximum anti-coagulant effect. Demonstration of the incubation period in this manner has not previously been reported. A review of the reported haparin clotting tests revealed that none showed the incubation effect, while only one[41] showed the logarithmic relation between heparin concentration and clotting time. These findings not only justify the technique employed in this test but provide a sound basis for its standardization and simplification.

Simplification of the Heparin Clotting Time for Clinical Use. The use of the 8 tube test is too time-consuming to be of value as a routine test in the clinic. It was found that by the use of only 2 of the tubes, 0 and 3, the test became extremely simplified and gave very adequate information. This is readily shown in Figure 6, which shows the normal range and results of the 8 tube test for 12 polycythemia vera and 8 leukemia patients. The significance of these findings will be discussed in Part II, but it can be noted that all but 4 of the leukemia patients show marked increases in clotting time for all the tubes and that Tube 3 gives as much information as any of the other tubes. Tube 3 has a convenient clotting time range of 22 to 40 minutes for normal subjects, which gives ample margin for detecting both increases and decreases. This clotting range was long enough to avoid the factor of the incubation period, which would markedly affect Tube 2. Tube 2 also does not offer much margin for the detection of increases in the clotting time. Use of the higher heparin concentrations would unnecessarily prolong the test and would also be less accurate since poorer end points occur with longer clotting times. Blood that did not clot within 400 minutes remained incoagulable.

The heparin prolonged clotting time or heparin clotting time will refer to the clotting time obtained for 1 cc. of venous blood to which .004 milligram of heparin has been added (Tube 3). In the two tube test, Tube 0 is examined for clotting every half-minute starting at three minutes, and Tube 3 is examined at one minute intervals starting at 15 minutes. Figures 7 and 8 show the distribution of the results of each tube for normal subjects and patients. The heparin clotting time for 33 normal subjects (16 males, 17 females) was as follows:

Average value: 27.5 minutes.

Range ( $\pm$ 2 standard deviations: 20.3 to 34.7 minutes

There was no significant difference between the average values for the males and females. Duplicate measurements on aliquot samples of blood showed less than 5 percent variation.

## C. Methods of the Other Tests

Clotting Time. Two variations were used. In the early part of the study, one-half cc. of blood was placed in each of two dry, glass tubes, 75 x 10 mm. The first tube was observed for clotting by tilting every one-half minute. When clotted, the second tube was then observed.

In the latter two-thirds of this study, a single dry, glass tube, 100 x 13 mm., was filled with 1 cc. of blood and observed for clotting every one-half minute after 3 minutes. This tube was identical with Tube 0 described above. Routine inspection was made for the presence of clot retraction at 4 and 24 hours. All measurements were made in a constant temperature water bath set at 37 degrees centigrade.

Bleeding Time. The method described by Duke[17] was used. The ear lobe was firmly punctured with a pointed scalpel blade and observed every one-half minute for stoppage of bleeding.

Prothrombin Time. The variation of the Quick[29] method described by Rosenfeld and Tuft[47] was used with a few modifications. Dried rabbit brain thromboplastin (made by Difco) was freshly prepared each time measurements were made. .0125 molar calcium chloride was used. Determinations were made on 100 percent and 10 percent plasma, which was diluted with barium sulfate treated plasma.

Tourniquet Test for Increased Capillary Fragility. A rubber tourniquet was snugly placed around the lower part of the arm for 5 minutes such that the arterial pulse was not obliterated. A positive test, indicative of increased capillary fragility, consisted of the appearance of numerous petechiae below the level of the tourniquet.

Hematocrit. Heparinized blood was placed in a Wintrobe tube and centrifuged for one-half hour at 2,000 revolutions per minute. The reading of the red cell volume in percent was then made.

Sedimentation Rate of Red Cells. Heparinized blood was placed in a Wintrobe tube and the amount of red cell sedimentation was read in mm. after one hour. Morrison's findings[48] of a close relation between the red cell sedimentation rate and clot retraction was not substantiated by our findings.

Toluidine Blue Titration Test. This test measured the effect of a series of concentrations of toluidine blue on the clotting time of recalcified oxalated plasma. Blood was mixed with .1 molar sodium oxalate, 9 parts to 1, and centrifuged for 15 minutes at medium speed in a small clinical centriguge. .1 cc. of plasma was added to each of 6 increasing concentrations of toluidine blue* in .05 cc. of isotonic saline, as indicated in Table III. .1 cc. of a .025 molar calcium chloride solution was added to each tube which was then examined every 15 seconds for clot formation. A control tube containing the saline without any toluidine blue was also used.

Platelet Count. Red cell pipette and Rees- Eckers diluting solution were used. The counts were determined on capillary blood.

Red cell counts, white cell counts and differentials were performed on capillary blood according to routine laboratory technique. A photoelectric method was used in the hemoglobin determinations.

The values of these tests for normal subjects are listed in Table I. The average value and range are given.

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* Toluidine blue O, made by National Aniline Division, Allied Chemical and Dye Corp., New York, N. Y. Total dye content 69 percent.

TABLE III

TOLUIDINE BLUE TITRATION TEST PERFORMED ON RECALCIFIED OXALATED PLASMA

| Amount of toluidine blue in saline added to plasma | J. S. Normal | H.M. Normal | B.E. Chronic myel. leuk. | P.Q. Chronic lymph. leuk. | M.A. Poly. vera | H.K. Poly. vera | E.D. Multiple myeloma | W.P. Secondary poly. |
|---|---|---|---|---|---|---|---|---|
| milligrams | | | | | | | | |
| 0.0000 | 3.0 | 3.5 | 2.5 | 3.2 | 4.5 | 4.0 | 3.5 | 3.0 |
| 0.0015 | 3.2 | 3.5 | 2.5 | 2.7 | - | - | - | - |
| 0.0031 | 3.5 | 4.2 | 2.5 | 4.0 | 5.2 | 4.5 | 3.7 | 3.0 |
| 0.0062 | 3.7 | 5.5 | 3.0 | 4.0 | 5.5 | 4.7 | 4.5 | 3.2 |
| 0.0125 | 4.2 | 7.0 | 4.0 | 4.5 | 6.0 | 5.7 | 4.7 | 3.5 |
| 0.0250 | 5.0 | 8.0 | 5.0 | 5.0 | 8.0 | 8.0 | 5.7 | 4.2 |
| 0.0500 | 8.2 | 10.0 | 8.0 | 16.0 | 11.0 | 12.0 | 9.2 | 6.0 |

## Part II. Results

### A. Plan of Study

The methods of the tests employed in this study have been described and two new tests have been presented and discussed. As shown in Table IV, tests were performed upon normal subjects and patients seen at Donner Clinic. At various stages of of the study, different groups of tests were employed. In the last two-thirds of the study, the following tests were routinely performed: clot retraction rate by electric resistance measurements, heparin clotting time, clotting time (100 x 13 mm. tube), and hematocrit. Bleeding time, tourniquet test, erythrocyte sedimentation rate and toluidine blue titration were performed in certain cases as indicated. The prothrombin time was employed only in the early stages of the study.

Each time blood was taken for coagulation tests on patients, platelet count, red and white cell counts, differential and hemoglobin were also performed. Unfortunately, platelet counts or other blood counting data were not obtained on the normal subjects.

Blood for coagulation tests was obtained by a dry syringe and 20 gauge needle. Care was taken to enter the vein immediately in order to minimize tissue juice contamination. Enough blood was obtained so that the first and last cc. were not used for clotting tests. Usually, 6 to 8 cc. of blood was enough for all the tests. Blood was usually taken in the morning and no attempt was made to regulate diet or obtain fasting specimens.

### B. Leukemia

Review of the Literature. Hemorrhage has long been a major therapeutic problem in leukemia. In acute leukemia and many cases of chronic leukemia, bleeding can be attributed to a marked depression of platelet count and function. In these cases, the usual tests for platelet function such as the presence of clot retraction, bleeding time and tourniquet test show abnormal results[9]. However, bleeding in the absence of a definite thrombocytopenia or abnormality of the above clotting tests, as is frequently observed in chronic leukemia, has never been adequately explained. On the other hand, a marked thrombocytopenia may sometimes be present without any sign or symptom of hemorrhage. Wintrobe[1] suggests the possibility that infiltration of the vascular walls by leukemic cells may increase their permeability and thus may be an important factor in the bleeding.

The platelets have been recognized to show certain trends in the various types of leukemia[49]. In acute leukemia, there is usually a marked thrombocytopenia, while in chronic lymphatic leukemia the platelets may be normal or slightly depressed. In chronic myelogenous leukemia, the platelets are frequently increased or normal. The level of platelets appears related to the general status of the patient, with a progressive thrombocytopenia indicative of a poor prognosis.

Material. The patients comprising the present study were all ambulatory and all except two had chronic leukemia. Table IV lists the number of patients with each type of leukemia.

TABLE IV

BLOOD COAGULATION TESTS PERFORMED ON NORMAL SUBJECTS AND PATIENTS

| Type of subject | Total | | Clot retraction rate-electrical resistance | | Heparin clotting time | | Clotting time 100 x 13 mm. glass tube | | Clotting time 75 x 10 mm. glass tube | | Bleeding time | | Prothrombin time | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S - number of subjects<br>O - number of times observed or tested | | | | | | | | | | | | | |
| | S | O | S | O | S | O | S | O | S | O | S | O | S | O |
| Normal | 64 | 70 | 53 | 56 | 33 | 34 | 38 | 40 | 27 | 28 | 18 | 18 | 24 | 25 |
| Polycythemia vera | 45 | 85 | 42 | 74 | 35 | 49 | 35 | 51 | 16 | 33 | 5 | 14 | 9 | 17 |
| Acute leukemia | 2 | 4 | 2 | 4 | 1 | 1 | 2 | 4 | 1 | 3 | 2 | 4 | 0 | 0 |
| Chronic myel. leukemia | 9 | 10 | 8 | 9 | 7 | 7 | 8 | 9 | 2 | 2 | 6 | 6 | 1 | 1 |
| Chronic lymphatic leukemia | 16 | 25 | 14 | 22 | 12 | 17 | 14 | 19 | 5 | 8 | 6 | 12 | 3 | 6 |
| Chronic eosinophilic leuk. (?) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lymphomas | 4 | 6 | 4 | 6 | 3 | 5 | 3 | 5 | 1 | 1 | 1 | 1 | 0 | 0 |
| Multiple myeloma | 2 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| Agnogenic myeloid metaplasia | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Secondary polycythemia | 2 | 5 | 2 | 3 | 1 | 1 | 1 | 2 | 2 | 3 | 0 | 0 | 1 | 2 |
| Other diseases | 6 | 7 | 6 | 6 | 4 | 4 | 4 | 4 | 2 | 3 | 1 | 1 | 2 | 3 |
| Total | 153 | 217 | 136 | 185 | 100 | 122 | 109 | 138 | 57 | 82 | 40 | 57 | 40 | 54 |

Results. The most striking finding was the marked prolongation of the heparin clotting time in one-half of the patients. This can be readily seen in Figures 6 and 8, which also indicate the magnitude of this increase in comparison to the normal subjects. Division of the patients into two groups on the basis of this test provided further information. Group 1 consisted of 11 patients with heparin clotting times in the normal range below 40 minutes, while Group 2 included 11 patients with values over 40 minutes. Table V lists the coagulation and hematological data for these patients.

In Group 1, where the heparin clotting times ranged from 26 to 40 minutes, none of the patients had any clinical evidence of bleeding except in one case in which there was vaginal bleeding but this was found to result from a uterine neoplasm. The clot retraction rates were well within the range of normal except for one patient who showed a slight increase. Platelet counts were all above 200,000.

In contrast, Group 2 showed heparin clotting times ranging from 44 to 155 minutes. 8 of the 11 showed clinical evidence of bleeding, the severity of which appeared related to the degree of increase of the heparin clotting time. This bleeding was not severe or serious except for 1 case. It was usually manifested as nose bleeds, bleeding gums or excessive bleeding after such procedure as a sternal puncture. Manifestations of skin purpura occurred in only 4 cases. All but 3 of these 11 patients had impaired clot retraction with clot retraction rates below the normal lower limit of 4 ohm-cm. per minute. 7 out of the 8 patients with bleeding showed the impaired clot retraction. Platelets were below 200,000 in 8 of the 11 patients. 6 out of the 8 patients with bleeding had platelet depression with counts between 110,000 and 180,000. In 6 of the patients with bleeding, the bleeding time was definitely prolonged in two cases, slightly prolonged in 1 and normal in 3. The tourniquet test was negative in 4 patients with bleeding. The clotting times (without heparin) were in the normal range for patients of both groups.

The groups showed differences in hematological findings. In Group 1 the red cell counts and hematocrits appeared to parallel the platelets by showing consistently higher values than in Group 2. The white cell counts were similar to both groups. However, definite differences in the course of the disease were found between the two groups. Follow-up as of January, 1949, revealed that 1 patient in Group 1 and 5 patients in Group 2 had died.

These findings suggested the presence in certain patients with leukemia of a coagulation defect characterized by an increased heparin clotting time, a decrease in clot retraction rate and a slight thrombocytopenia. This coagulation defect appeared definitely related to the presence of bleeding and both the clinical and hematological status of the patient. The presence of this defect seems to be consistent with a poorer prognosis.

The electric resistance curves for these patients showed certain variations. Most of the patients showed the typical curve pattern which has been described for the normal subjects. There were, of course, differences in the initial resistance level and slope of the curve depending upon the hematocrit and degree of clot retraction. Curves of 4 leukemia patients are shown in Figure 9. The two cases of chronic lymphatic leukemia can be noted to have diminished clot retraction.

Atypical Electric Resistance Curve. Another group of patients, however, had curves whose pattern differed from the typical curve. Such atypical electrical resistance

TABLE V

COAGULATION AND OTHER DATA FOR LEUKEMIA PATIENTS

| Subject | Sex | Type of leukemia | Date examined | Heparin clotting time | Clinical evidence of bleeding | Clot retraction rate | Clotting time (no heparin) | Platelet count | Bleeding time | Red cell count | Hematocrit | White cell count (c.mm.) | Status on 1-49 as of (D-dead, L-living) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | ohm-cm per min. | min. | x10$^3$ | min. | x10$^6$ | | | |
| | GROUP I Heparin Clotting below 40 Minutes | | | | | | | | | | | | |
| GS | F | CML | 6-48 | 26 | 0+ | 10.2 | 8 | 200 | 8 | 4.70 | 44 | 17,300 | L |
| EW | F | CML | 4-48 | 27 | 0 | 8.6 | 6 | 520 | 2-1/2 | 4.00 | 35 | 44,500 | D 6-48 |
| WM | M | CLL | 4-48 | 28 | 0 | - | 9 | 290 | - | 5.74 | 56 | 60,000 | L |
| VC | F | CLL | 10-48 | 29 | 0 | 12.3 | 6-1/2 | 360 | - | 3.62 | 40 | 69,000 | L |
| RS | F | CML | 10-48 | 32 | 0 | 7.7 | 8-1/2 | 280 | - | 4.28 | 43 | 60,000 | L |
| GH | M | CLL | 12-48 | 34 | 0 | 9.0 | 7-1/2 | 220 | - | 4.60 | 45 | 27,800 | L |
| AJ | M | CLL | 10-48 | 35 | 0 | 5.4 | 9 | 200 | - | 4.84 | 47 | 54,800 | L |
| GH | F | CLL | 9-48 | 38 | 0 | 7.0 | 8-1/2 | 210 | - | 4.16 | 39 | 25,300 | L |
| WH | M | CEL | 9-48 | 38 | 0 | 7.5 | 8-1/2 | 200 | - | 3.80 | 41 | 34,000 | L |
| IJ | F | CML | 10-48 | 40 | 0 | 9.1 | 6-1/2 | 360 | - | 3.50 | 36 | 125,500 | L |
| | GROUP II Heparin Clotting Time over 40 Minutes | | | | | | | | | | | | |
| LW | M | AL | 4-48 | 44 | + | 3.6 | 6 | 300 | 2 | 3.12 | 34 | 20,500 | D 5-48 |
| LB | F | CML | 10-48 | 47 | ++ | 1.9* | 6 | 230 | 3-1/2 | 2.08 | 20 | 54,000 | D 11-48 |
| HW | M | CLL | 4-48 | 53 | 0 | 5.1 | 8 | 120 | 5 | 4.46 | 43 | 21,900 | L |
| TP | M | CLL | 6-48 | 58 | 0 | 6.8 | 6 | 300 | - | 4.10 | 41 | 16,800 | L |
| MM | M | CLL | 9-48 | 58 | 0 | 2.4* | 8 | 160 | 3 | 2.90 | 29 | 10,300 | L |
| PB | M | CLL | 5-48 | 62 | + | .6 | 5-1/2 | 180 | - | 3.80 | 32 | 37,000 | D 8-48 |
| CS | F | CLL | 6-48 | 118 | + | 4.7 | 8 | 160 | - | 3.74 | 38 | 29,750 | L |
| RJ | F | CML | 9-48 | 122 | ++ | .8* | 9-1/2 | 130 | 20 | 2.10 | 20 | 7,950 | L |
| JC | M | CLL | 4-48 | 130 | ++ | 1.6 | 7 | 180 | 5 | 3.40 | 36 | 8,100 | D 7-48 |
| BE | M | CML | 4-48 | 154 | +++ | .5* | 4 | 110 | 11 | 1.73 | 20 | 90,000 | D 5-48 |
| JH | M | CLL | 4-48 | 155 | ++ | .1* | 7 | 160 | 8 | 2.80 | 27 | 30,000 | L |

\+ Vaginal bleeding, subsequently discovered to be caused by uterine carcinoma.

* Atypical electric resistance curve.

AL - Acute leukemia
CML- Chronic myelogenous leukemia
CLL- Chronic lymphatic leukemia
\+ Slight ++ Moderate +++ Marked bleeding

curves are illustrated and explained in Figure 10. This was the only variation from the typical pattern encountered. Patients with this finding generally had low red cell counts, low hematocrits and depressed platelet counts. They showed rapid red cell sedimentation which produced an increase in the red cell concentration around the electrodes before clotting occurred. This, in turn, gave an "initial resistance increase" which continued until the blood formed a solid clot which prevented further sedimentation. This was followed by a period of unchanged resistance, after which the resistance again increased because of clot retraction.

Interpretation of these curves was complicated by the fact that the sedimentation caused a lack of uniformity in the red cell distribution in the clot. The reason for the varying period of unchanged resistance appeared related to a delay in the start of clot retraction in the electrode area where the increased red cell concentration would lead to a displacement of serum and platelets. This would give a relative deficiency of fibrin and platelets in the clot around the electrode area and in turn result in diminished clot retraction. However, clot retraction rates, taken from the second resistance increase as indicated in Figure 10, have been compatible with the other coagulation and hematological findings in each case.

In Table V the clot retraction rates obtained from atypical curves are indicated. These rates ranged from .1 to 2.4 ohm-cm. per minute, indicative of impaired retraction. Among 9 patients showing an atypical curve, 6 had clinical signs of bleeding.

In 10 cases where inspection up to 24 hours revealed absent or very slight clot retraction, electric resistance measurement provided quantitative data indicative of some degree of retraction in 6 cases. The other 4 showed flat curves as in Figure 10, patient J.H., 4/16/48.

Toluidine Blue Titration. Recent literature describes the discovery of free heparin or heparin-like anticoagulants in the blood of dogs after lethal doses of total body x-irradiation[50] and in human subjects following nitrogen mustard therapy[51]. There are reports of beneficial effects of toluidine blue upon hemorrhage in cases of thrombocytopenic purpura[38,52]. In view of this, a toluidine blue titration test for heparin was performed upon two patients with marked hemorrhagic symptoms. One patient, B.E., whose findings are listed in Table V, had a heparin clotting time of 154 minutes, a markedly depressed clot retraction rate of .5 ohm-cm. per minute, 110,000 platelets, and bleeding time of 11 minutes. The other patient, P.Q., chronic lymphatic leukemia, had absent clot retraction by electric resistance, platelets 140,000 (none seen 2 months later), and bleeding time over 15 minutes. Neither patient showed the presence of free or neutralizable heparin. This test was also negative for free heparin in 2 normal subjects, 2 patients with polycythemia vera, 1 with multiple myeloma and 1 with polycythemia secondary to a congenital cardiac defect. These results are shown in Table III.

Coagulation Studies as an Aid in Surgery of the Leukemic Patient. One case illustrates the value of these coagulation studies in the diagnosis of the etiology of bleeding and the prediction of the possibilities of hemorrhage in surgery. This patient, G.S., 62 years old, has had chronic myelogenous leukemia for 6-1/2 years. When seen, she had been having vaginal bleeding for two weeks. This was the first vaginal bleeding that had occurred since she received x-ray to the ovaries 10 years

previously. There was no other evidence of bleeding. Coagulation studies revealed: heparin clotting time 26 minutes, clot retraction rate 10.2 ohm-cm. per minute, platelets 200,000, clotting time 8 minutes and bleeding time 8 minutes. Hematological findings are shown in Table V and her electric resistance curve is illustrated in Figure 9 d. These tests showed completely normal coagulation function and indicated the probability of local pathology as the etiology of the bleeding. A dilatation and curretage revealed a uterine carcinoma for which surgery was performed. During surgery and post-operatively the patient showed no abnormal bleeding and made a satisfactory recovery.

## C. Polycythemia Vera

Review of the Literature. The general opinion upon coagulation, thrombosis and hemorrhage in polycythemia vera has been well summarized by Harrop and Wintrobe[53]: "... The blood platelets may be normal in number but are frequently increased, sometimes to three or four times their normal values. ...The various tests of the efficiency of blood coagulation reveal no striking abnormality. Bleeding time is usually essentially normal and coagulation time normal. In spite of thrombocytosis clot retraction may be slow and little serum is usually formed, probably because of the great increase in the size of the blood mass. These features may explain not only the abnormal tendency to thrombosis in this disease but the blood vessel engorgement and slow clot retraction suggest an explanation for the frequent hemorrhages."

It has also been observed that the clot forming from polycythemic blood is fragile and may dissolve or become fluid upon minimal agitation[54].

Material. This study is based upon observations on 45 patients with definite polycythemia vera seen at Donner Clinic. The majority of these patients were seen before tre tment while their red cell counts and hematolocrits were greatly elevated. Two of the patients were in the leukemic phase of polycythemia. None of the patients showed active thrombosis or hemorrhage during the time of observation.

Results. The results are summarized in Table VI. More than one-third of the untreated patients had an elevation in platelet count and increase in clot retraction rate. In the heparin clotting time test, most of the patients were in the normal range. 33 percent of all the patients had histories of either thrombosis or hemorrhage. Distribution of the clot retraction rates with a comparison to those for normal subjects is shown in Figure 3. Figures 6 and 8 show the results of the heparin clotting test.

In the clot retraction studies, inspection of the tubes frequently showed poor retraction, while at the same time the electric resistance curve indicated abnormally rapid retraction. When the electrodes were removed after 24 hours, very small firm clots were often found retracted between the electrodes. The red cells suspended in serum accounted for most of the volume of the sample. In patients with very high hematocrits, end points of clotting were difficult to obtain. A solid clot occasionally never formed.

Division of the polycythemia patients into two groups, those with platelet counts above 400,000 and those below 400,000, revealed marked differences in the average clot retraction curve for each group. These curves gave clot retraction rates of 11.0 and 7.3 ohm-cm. per minute respectively, as illustrated in Figure 4. Figure 12 shows resistance curves for 4 polycythemia patients. The two upper curves show rapid clot retraction, while the lower two are examples of normal retraction.

TABLE VI

SUMMARY OF BLOOD COAGULATION RESULTS IN POLYCYTHEMIA VERA

| Group | Number of Patients | Elevated Hematocrit | History of | | Platelet Count | | | Clot retraction rate | | | Heparin clotting time | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | bleeding | thrombosis | low | normal range | high | low | normal range | high | low | normal range | high |
| Untreated cases | 39 | 39 | 7 | 6 | 0 | 25 | 14 36% | 0 | 24 | 12 34% | 3 | 21 | 6 |
| Treated-in remission | 4 | 0 | 1 | 0 | 1 | 3 | 0 | 0 | 4 | 0 | 0 | 2 | 2 |
| Leukemic phase | 2 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 |

Coagulation and hematological data for the 12 patients with increased clot retraction rates are listed in Table VII. 9 of these patients had platelet counts above 400,000, and only 1 had a prolongation in heparin clotting time. None of these patients had received treatment, except for phlebotomy, and all had high red cell counts and hematocrits.

Possible relation between the hematocrit and heparin clotting time merits consideration. Since the heparin is added to a constant volume of blood, samples with higher hematocrits will have a proportionately higher heparin concentration in the plasma. As the red cells have not been shown to play any active part in coagulation, patients with high hematocrits could be expected to have an increased heparin clotting time because of the higher heparin concentration in their plasma. This possibility was found to be true for one patient, M.A., who was tested on four different occasions as his hematocrit decreased in response to treatment with radioactive isotopes. As shown in Figure 13, the clotting times for the 8 tube heparin clotting test differed markedly on each occasion when plotted according to heparin concentration in blood. When plotted against heparin concentration in plasma, the results fell close to a straight line. Correction of the heparin clotting time for hematocrit differences, changed the distribution of results such that 5 patients were transferred from the normal range to below the normal. Thus, 6 out of 10 patients with increased clot retraction rates, had decreases in the heparin clotting time. As shown in Table VIII, a few of the polycythemia patients showed remarkably little variation in heparin clotting time over intervals of many months.

Bleeding and prothrombin times were found to be normal.

## D. Other Patients

Because the number of patients in this group is small and varied, only a few general observations are mentioned. Table IV lists the diseases of patients in this group, which can be divided into those without primary hematopoietic disease and those with primary hematopoietic disease including the lymphomas. Patients in the latter group usually had some bone marrow depression with anemia and slight thrombocytopenia. None had hemorrhagic symptoms. The other group showed normal blood findings except for 2 patients with secondary polycythemia. In Figure 3, it is of interest to note that the patients without primary hematopoietic disease show a distribution of clot retraction rate similar to the normal subjects and the other patients with hematopoietic disease or lymphoma show lower clot retraction rates. Similar differences were noted for the heparin clotting time, with patients with hematopoietic disease showing increases (Figure 8). The clotting times (without heparin) were all in the normal range (Figure 7).

The coagulation and hematological findings for H.P., giant follicular lymphoblastoma, on three different occasions showed definite consistency as shown in Table VII. Figure 9,e and f, shows the clot retraction curves for patients with agnogenic myeloid metaplasia and multiple myeloma.

## E. Effect of Treatment with Radioactive Isotopes on Coagulation

Although study on the effect of radioactive isotopes upon coagulation is still in progress, some preliminary observations may be made. The radioactive isotopes do not appear to have any direct or immediate effect on any of the coagulation tests.

TABLE VII

COAGULATION AND HEMATOLOGICAL DATA OF POLYCYTHEMIA VERA PATIENTS WITH INCREASED CLOT RETRACTION RATE

| Patient | Sex | Date | Clot retraction rate | Platelet count | Heparin clotting time | Clotting time | Red cell count | Hematocrit | White cell count |
|---|---|---|---|---|---|---|---|---|---|
| | | m-y | ohm-cm. per min. | cu.mm. | min. | min. | cu.mm. | % | cu.mm. |
| A.F. | F | 10-47 | 10.2 | 680,000 | - | 9 | 5.72 | 45 | 18,800 |
| H.M. | M | 9-48 | 10.3 | 200,000 | 29 | 9 | 7.34 | 66 | 16,600 |
| L.G. | M | 4-48 | 10.5 | 600,000 | 26 | 7 | 8.16 | 69 | 14,200 |
| A.P. | M | 10-47 | 11.1 | 470,000 | - | 8 | 7.70 | 58 | 21,800 |
| H.K. | M | 4-48 | 12.3 | 350,000 | 31 | 6 | 5.54 | 51.5 | 13,500 |
| T.F. | M | 6-48 | 13.2 | 400,000 | 25 | 6 | 5.12 | 52 | 12,500 |
| E.W. | F | 10-48 | 13.9 | 480,000 | 25 | 11 | 6.84 | 60 | 39,000 |
| E.B. | M | 11-48 | 13.9 | 580,000 | 20 | 8 | 8.30 | 61 | 33,000 |
| G.T. | M | 6-48 | 15.0 | 376,000 | 17 | 5 | 6.16 | 65 | 12,000 |
| E.K. | F | 10-48 | 15.1 | 490,000 | 43 | 10 | 7.45 | 62.5 | 10,300 |
| C.R. | M | 10-48 | 15.7 | 540,000 | 24 | 9 | 6.88 | 64 | 35,500 |
| F.T. | M | 11-48 | 17.6 | 580,000 | 24 | 8 | 6.42 | 56 | 13,750 |

The isotopes do appear to have an effect on coagulation insofar as they affect either the platelets or the general condition of the patient. Some illustrative cases are briefly described.

Patient J.H. was treated for chronic lymphatic leukemia with radioactive isotopes and a few x-ray treatments and showed marked clinical and hematological improvement. As shown in Figure 11, his clotting tests improved, especially the heparin clotting time which dramatically changed towards normal from 155 to 45 minutes. The clot retraction rate increased slightly and symptoms of bleeding disappeared. The platelets showed no significant change. The clot retraction curves are shown in Figure 10.

A few cases have shown prolonged heparin clotting times and lower clot retraction rates accompanied by platelet depression following radioactive isotope therapy. This occurred in B.L., polycythemia vera, listed in Table VIII.

In patients where the isotopes have produced no definite change in either the general condition, blood picture or platelet count, no alteration has been detected in the coagulation tests. This is demonstrated by patients E.L., S.S. and H.P., shown in Table VIII. During the one and one-half year span of this study, no hemorrhagic complications or thrombocytopenias below 80,000 have occurred following and attributable to radioactive isotope therapy.

Certain patients studied at intervals of several months have had remarkably little variation in results of the coagulation tests as shown in Table VIII. This is true only for cases where the clinical and hematological status also remains about the same.

## F. Statistical Analysis of Data

A statistical study of the possible relations between the various tests assists in understanding the results which have been presented. Table IX shows the tests compared, their correlation coefficients and standard deviations. The dangers of placing undue emphasis upon such statistical analyses should be remembered. The interpretations of the degree of correlation are based upon Guilford's discussions[55]. A positive correlation coefficient indicates a direct relation; a negative an inverse relation. For perfect or 100 percent correlation, the coefficient (r) would be 1.00.

Clot Retraction Rate. This test shows a high correlation with platelet count and a slightly lower correlation with hematocrit. However, the platelets and hematocrit also show a moderate correlation. This reflects the nature of the type of patients comprising this study. The untreated polycythemic patients had both high hematocrits and high platelet counts, while leukemic patients with low platelet counts usually had low hematocrits. Study of individual cases indicated a closer relation between the clot retraction rate with platelet count rather than with hematocrit. In any quantitative comparison involving the platelets, the large error inherent in any method of platelet counting must be considered.

A difference between the clotting time (without heparin) and the time of the first resistance increase is suggested by a comparison of their relations to the clot retraction rate. The clotting time shows no correlation with the clot retraction rate. However, the time of first resistance increase shows a low, significant correlation with the clot retraction rate. This indicates that clot retraction may

TABLE VIII

COAGULATION AND HEMATOLOGICAL DATA AND RADIOACTIVE ISOTOPE TREATMENT OF PATIENTS STUDIED ON DIFFERENT OCCASIONS

| Patient and diagnosis | Date | Heparin Clotting Time | Clotting time | Clot retr. rate | Platelet count | Red cell count | Hematocrit | White cell count | Radioactive isotope therapy previous 4 weeks |
|---|---|---|---|---|---|---|---|---|---|
| | m-d-y | min. | min. | Ohm-cm./min | cu.mm. | mil./cu.mm. | % | cu.mm. | MC-millicurie |
| G.H. Chr.lymph. leukemia | 3-24-48 | 28 | 6.5 | - | 390,000 | 4.89 | 45.5 | 46,000 | None |
| | 12-2-48 | 34 | 7.5 | 9.0 | 220,000 | 4.60 | 45 | 27,800 | None |
| T.P. Chr.lymph. leukemia | 6-8-48 | 58 | 6.0 | 6.8 | 300,000 | 4.10 | 40.5 | 16,800 | None |
| | 10-18-48 | 58 | 10.5 | 7.8 | 260,000 | 4.76 | 44 | 9,700 | None |
| E.L. Poly. vera | 5-26-48 | 23 | 7 | 9.9 | 300,000 | 6.02 | 55.5 | 11,300 | 3 MC of |
| | 5-28-48 | 30 | 7 | 9.6 | 300,000 | 5.54 | 55.5 | 12,400 | $P^{32}$ on |
| | 6-4-48 | 25 | 6 | 7.9 | 280,000 | 5.70 | 53.5 | 9,800 | 5-27-48 |
| | 6-10-48 | 31 | 7 | 9.0 | 380,000 | 5.58 | 52 | 10,500 | |
| B.L. Poly. vera | 4-1-48 | 27 | 8 | - | 300,000 | 6.89 | 59.5 | 16,700 | 3 MC of |
| | 4-28-48 | 58 | 9 | 4.4 | 190,000 | 5.28 | 58 | 12,900 | $Y^{90}$ on |
| | 10-19-48 | 36 | 7.5 | 8.0 | 230,000 | 3.85 | 46 | 9,500 | 4-1-48 |
| W.W. Poly. vera | 6-15-48 | 32 | 11 | 8.3 | 280,000 | 6.81 | 62 | 8,700 | None |
| | 11-7-48 | 31 | 6 | 4.8 | 260,000 | 4.09 | 42 | 8,800 | None |
| S.S. Poly.vera | 3-24-48 | 21 | 5 | - | 350,000 | 3.80 | 40 | 57,500 | 3 MC of $P^{32}$ |
| Chr.myel. leukemia | 12-2-48 | 24 | 6 | 7.2 | 320,000 | 4.00 | 45 | 56,000 | None |
| H.P. Giant fol. lymphobl. | 4-27-48 | 40 | 7 | 1.5 | 160,000 | 3.60 | 31 | 4,700 | 1 MC of $P^{32}$ |
| | 6-8-48 | 41 | 5 | 0.2 | 160,000 | 3.89 | 32.5 | 3,400 | 1 MC of $P^{32}$ |
| | 9-24-48 | 36 | 9.5 | 2.2 | 140,000 | 3.56 | 33 | 2,100 | .5 MC of $P^{32}$ |

TABLE IX

STATISTICAL ANALYSIS OF BLOOD COAGULATION DATA

| TESTS COMPARED | Number of subjects | Corr. coef. (r) | St. dev. ($\sigma$) | Degree of corr. |
|---|---|---|---|---|
| Clot retraction rate and platelets | 82 | + .73 | .05 | High |
| Clot retraction rate and hematocrit | 82 | + .68 | .06 | Moderate |
| Platelets and hematocrit | 82 | + .56 | .08 | Moderate |
| Clot retraction rate and time of first res. increase | 90 | - .36 | .09 | Low |
| Clot retraction rate and clotting time | 90 | + .11 | .11 | None |
| Initial res. and hematocrit | 82 | + .84 | .03 | High |
| Heparin clotting time and clot retraction rate | 64 | - .56 | .09 | Moderate |
| Heparin clotting time and platelets | 65 | - .45 | .10 | Moderate |
| Heparin clotting time and hematocrit | 65 | - .34 | .11 | Low |
| Heparin clotting time and clotting time (without heparin) | 65 | - .11 | .11 | None |

start before a solid clot is formed. This was substantiated by actual observation.

The initial level of electric resistance and hematocrit show an expected high correlation[27].

Heparin Clotting Time. This test shows moderate correlation with the clot retraction rate and platelet count, low correlation with the hematocrit, and no correlation with the clotting time (without heparin). This is suggestive of some relation between platelet function and heparin effect upon coagulation as will be discussed later.

## Discussion

Two important questions are suggested by this study: What factors influence the coagulation tests, especially the heparin clotting time and the clot retraction rate? What is the significance of the coagulation abnormalities detected by these tests in this group of patients? The discussion is based upon these questions, whose eventual answers may lead to effective prevention and treatment of hemorrhage and thrombosis.

The accuracy and usefulness of any information depend upon the methods employed in obtaining it. This is particularly true of blood coagulation. A review of the few coagulation tests which have been most widely used in clinical medicine reveals them to be of limited value, subject to excessive error and inconsistency. The platelet count[56], capillary fragility test[57] and the bleeding time, which are liable to both subjective and inherent errors[10], give only qualitative information at best. The clotting time (without heparin) does give consistent results for any one modification. However, only a very small fraction of cases, even where there is a bleeding problem, show any abnormality of this test[58,59]. Hemophilia is the most prominent condition where a prolonged clotting time is found. Figure 7 shows the distribution of clotting times in the present study. They were all within the normal range. The limited value of this widely used test cannot be over-emphasized. The prothrombin time shows significant alterations in limited types of hemorrhagic dyscrasias[29,60].

The heparin clotting test and the clot retraction rate as measured by electric resistance have been presented and described in detail earlier in this paper. They fill the clinical requirements of coagulation tests by giving consistent and accurate data of a quantitative nature. They appear to provide useful information on the true coagulation status of a patient. They have a well defined normal range which allows adequate margin to demonstrate either increases or decreases in value.

The use of heparin in coagulation tests not only gives valuable diagnostic information but may help to solve problems concerning physiological and pathological functions of heparin. There are indications of involvement of these functions in both hemorrhagic and thrombotic conditions. This is suggested by a few reports of the beneficial effect of toluidine blue, a heparin antagonist, in certain patients with hemorrhage[38,52] and the good results obtained by anticoagulant therapy with heparin and dicumarol in thrombotic conditions[61-66].

Since its discovery, heparin has been considered the "physiological anticoagulant" formed in the body for the purpose of keeping the blood fluid and partially regulating the different factors which inhibit or promote coagulation[67]. Although these functions have yet to be demonstrated, numerous facts concerning the chemistry of heparin[61,62] some mechanisms of its anticoagulant action[68-72] and its relation to mast cells[61,73,74] have been revealed. Great interest[75] in heparin has been stimulated by reports of its

appearance in excess in the blood under certain circumstances; following large doses of total body X-irradiation[50], nitrogen mustard administration[51], and anaphylactic and peptone shock[73,76,77,78,79]. There appear to be species differences in the degree and occurrence of this reaction. The dog readily liberates great amounts of heparin into its blood after anaphylactic or peptone shock or total body x-irradiation. This reaction has been found to be absent or minimal in the rabbit, rat and mouse[80-85]. A species difference has also been reported in the anticoagulant potency of heparins. Dog heparin shows by far the most activity. All the heparins showed chemical similarity and the same base binding ability such as combination with toluidine blue or protamine sulfate[86].

The question of the part played by heparin in hemorrhagic and thrombotic conditions has remained unanswered for a long time because of the limitation of methods to study heparin and its physiology. It has been impossible to detect heparin or heparin-like substances in a normal blood sample by chemical or biological means. Apparently, the small amount of heparin released into the blood under normal conditions conbines with basic substances and loses it anticoagulant properties. The most satisfactory method at present for the study of heparin appears to be the measurement of the effect of added heparin on clotting time. Use of this technique to demonstrate hypercoagulability in patients with thromboses has been mentioned. This technique has had limited general use because of its inadequate standardization and simplification. The heparin clotting test presented in this paper has been designed to overcome these deficiencies.

The effect of heparin on the clotting time after its addition to the blood probably involves two main factors or reactions: 1) inactivatiom or neutralizatiom of heparin by its combination with basic substances; 2) inhibition of the clotting mechanism by heparin. It is well known that heparin, a strong acid, will combine with many basic substances and thereby lose its anticoagulant action[87-91]. The use of protamine or toluidine blue to identify heparin by the formation of inactive complexes with it depends upon this property. Heparin may also react in a similar manner with components of the blood such as proteins. Heparin which is not combined and thus inactivated will act upon the clotting mechanism as an antagonist to thromboplastin, antiprothrombin and antithrombin[68-72]. The relative importance and exact nature of these factors is not known.

The significant prolongation in the heparin clotting time reported in this paper for patients with leukemia and other hematopoietic diseases cannot be explained. This coagulation defect appears related to bleeding symptoms, diminished clot retraction rate and slight thrombocytopenia (100,000 - 200,000). These relations appear to be quantitative in that patients with more severe bleeding had longer heparin clotting times and lower clot retraction rates. It should be emphasized that most of the patients included in this study did not have marked purpura, hemorrhages or severe thrombocytopenia (below 100,000). The toluidine blue titration test in 2 patients with severe hemorrhages revealed no free or neutralizable heparin in the blood.

Leukemia patients without any evidence of bleeding showed normal heparin clotting times, normal clot retraction rates and platelets over 200,000. These patients appeared to be in better condition, both hematologically and clinically. As of January, 1949, only one of the ten patients in this group had died, while among the patients showing the coagulation defect, five of eleven had died.

This indicates that an increased clotting sensitivity to heparin is associated with some impairment of platelet function as evidenced by diminished clot retraction rate and slight thrombocytopenia. The appearance of excess heparin in the blood following total body X-irradiation, nitrogen mustard therapy, anaphylactic or peptone shock has been invariably accompanied by marked decreases in the platelet count[50,51,93,94]. There are few investigations of the relation between heparin and platelets. The degree of clotting time prolongation of recalcified plasma by heparin is found to vary inversely with the number of platelets present[33]. Injection of heparin into certain mammalian species produces a rapid decrease in platelet count and marked platelet agglutination[95-98]. The dog reacts markedly in this manner, while in man and in rabbit, this reaction is negligible[84]. This finding parallels the species difference mentioned above, between the dog and rabbit in showing the hyperheparinemia reaction.

A distinction must be made between platelet count and platelet function. Discrepancies frequently have been noted among clinical symptoms of bleeding, results of coagulation tests and platelet counts[9,10,56]. This suggests variations in platelet function which cannot be detected by morphological appearance or counts. Platelet functions have been classified as follows[99]: 1) coagulative 2) agglutinative 3) adhesive 4) retractive 5) pressor 6) hemostatic. These groups are inter-related but they form a basis for the study of platelet function and their possible relations to heparin. Recent work indicates that platelets are involved in the coagulation mechanism in several different ways[100,101]. Changes in agglutination and adhesive properties have been demonstrated in both thrombotic and hemorrhagic conditions[102,103,104]. The present study has revealed quantitative differences in the degree of clot retraction in leukemia, polycythemia and other hematopoietic diseases.

It is probable that pathologic conditions and agents which cause alterations in the platelet count also cause variations in their functional properties. One function of the platelets may be responsible for counteracting or balancing the effect of heparin normally released into the blood. This function may involve either inactivation of heparin by combining with it or by supplying a coagulation accelerator which counteracts the anticoagulant action of heparin. In disease states where added heparin results in either increased or decreased clotting reaction changes in either platelet count or function have been reported. For example, increases in platelet counts, adhesiveness and agglutination have been found in certain thrombotic conditions which also show a decreased clotting sensitivity to heparin. In the present study, patients with decreased platelet counts and impaired clot retraction have been shown to have an increased clotting sensitivity to heparin as shown by prolongation in heparin clotting time.

The suggestion[97] that platelet changes are secondary to a heparinemia following total body X-irradiation seems unlikely as similar platelet alterations are seen without any evidence of excess heparin in the blood. The role of the mast cells, which probably produce heparin, warrants study. It is probable that variations in the amount of heparin released into the blood under abnormal circumstances contribute to the problem. It appears, however, that the platelets, unless proven otherwise, play an important part in heparin physiology. Further investigation is necessary to determine all the reactions involved when heparin is added to the blood. Knowledge of these reactions may lead to new and effective means of prevention and treatment of hemorrhagic and thrombotic conditions.

Blood Coagulation in Polycythemia & Leukemia
Relation of Heparin and Platelets; Quantitative Measure
of Clot Retraction & Heparin Clotting Time.

1. Two test of blood coagulation. Heparin clotting time. Clot retraction time.
(electrical resistance)

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In polycythemia vera, there were increases in the clot retraction rate and platelet count in over 33 percent of the untreated patients. This evidence of increased platelet function probably contributes to the frequency of thrombisis in this disease. At the same time, it was found that patients with high hematocrits formed fragile, rapidly dissolving clots which appeared to have poor retraction. However, electric resistance measurements and careful examination often indicated the presence in the sample of a small, well retracted clot surrounded by a red cell suspension in serum. This defective clot formation probably contributes to the tendency towards bleeding in this condition. With a relatively small amount of plasma present, a wider latticed fibrin network clot is formed. Under the influence of a large number of platelets, retraction occurs at a rapid rate but fewer red cells are caught in the network so that the retracted clot becomes very small, leaving most of the red cells suspended in serum at the periphery. The relatively small amount of serum available to displace to the periphery helps account for the appearance of poor retraction.

The heparin clotting time was normal in 24 cases of polycythemia vera, decreased in 3 cases and increased in 8 cases. The increase in one case was found to be the result of the high hematocrit which gave a proportionately high heparin concentration in the plasma. After correction was made for hematocrit, a total of 8 patients showed heparin clotting times shorter than normal. In this series of 45 patients, 33 percent had a history of either thrombosis or hemorrhage. For the prevention of these complications, the necessity of treatment which reduces both the increased platelet count and function is clearly indicated.

Some observations upon the effect of radioactive isotope therapy have been made. It is important to note that this type of treatment does not necessarily lead to impairment of blood coagulation but may often produce improvement in a clotting defect. This is shown in a few cases in this study. There is minimal danger of producing a serious hemorrhagic complication if the radioactive isotopes are carefully used. On the other hand, the depressant action of radioactive isotopes upon platelets enhances their use as therapeutic agents in polycythemia vera.

## Summary

1. Two tests of blood coagulation are presented. They are the heparin clotting time and the clot retraction rate, a quantitative measure of clot retraction obtained by electric resistance measurements. These tests, standardized on 64 normal human subjects, gave well defined normal ranges of 20 to 35 minutes for the heparin clotting time and 4 to 10 ohm-cm. per minute for the clot retraction rate. The results in this study suggest both the diagnostic and experimental value of these tests. The heparin clotting time is easy to perform and is recommended for routine clinical use. The clot retraction rate, which requires special equipment to perform, appears to be of particular value in investigations of platelet function. Some observations on clot retraction and effect of heparin on the clotting time are presented.

2. The limitations and inadequacies of the various coagulation tests now in widespread clinical use are discussed.

3. A review of the literature revealed no adequate studies of blood coagulation in polycythemia vera or chronic leukemia, two diseases in which thrombosis and hemorrhage are serious complications and therapeutic problems.

4. The results of various coagulation tests upon 89 patients are reported.

a. In chronic leukemia, either myelogenous or lymphatic, one-half of the patients showed a previously unreported coagulation defect characterized by a prolongation of the heparin clotting time, decreased clot retraction rate and a slight thrombocytopenia between 110,000 and 180,000. 8 of 11 patients showing this defect had some clinical evidence of bleeding. No free or neutralizable heparin was detected by a toluidine blue titration test in the blood of 2 patients with marked hemorrhagic symptoms. Leukemia patients without this defect and thus having normal values for the coagulation tests showed no evidence of bleeding. Patients showing the coagulation defect were in poorer clinical and hematological condition and had a poorer prognosis as 5 out of 11 had died, whereas only 1 out of 10 patients with normal coagulation had died in the same period.

b. 45 patients with polycythemia vera were studied. Of the untreated patients with elevated red cell counts and hematocrits, 33 percent showed increased clot retraction rates and platelets elevated above 400,000. On inspection, the clots were often found to be fragile and poorly retracted. An explanation for the discrepancy between the rapid clot retraction rate and external appearance of poor retraction in identical samples of blood is given. The heparin clotting time was generally normal with 8 patients showing prolongations and 3 decreases. When corrected for the evelated hematocrit, 8 out of 35 patients had heparin clotting times shorter than normal.

c. A coagulation defect similar to that found in certain leukemia patients was also seen in some patients with other hematopoietic diseases. Patients without any primary hematopoietic disease gave normal coagulation results.

5. Statistical analysis of the data showed a high correlation between the clot retraction rate and platelet count. There were moderate correlations between the heparin clotting time and both the clot retraction rate and the platelet count.

6. The possible relations of platelets and heparin to various hemorrhagic and thrombotic conditions are discussed. An analysis of the literature revealed that in all conditions which show altered clotting reactions to heparin or free heparin in the blood, functional abnormalities of the platelets appear to be invariably present. This relation was found to be present in this study. The platelets and heparin seem to be vital factors in blood coagulation alterations which are responsible for various hemorrhagic and thromo-embolic complications. Understanding of these factors and alterations should lead to effective means of diagnosis, prevention and treatment of many hemorrhagic and thrombotic conditions.

7. Preliminary observations indicate that radioactive isotope therapy in leukemia and polycythemia has beneficial effects upon altered blood coagulation, in conjunction with clinical and hematological improvement.

The author wishes to thank Dr. John H. Lawrence for his valuable guidance and continued interest. The assistance of Agnes Benedek is gratefully acknowledged. He is grateful to Drs. C. A. Tobias, H. Jones, C. W. Tobias, J. Weaver, L. Wasserman and many other people at Donner Laboratory for their kind cooperation and assistance.

BIBLIOGRAPHY

1. Wintrobe, M. M.: Clinical Hematology, Lea & Febiger, Philadelphia, 1946.

2. Norman, I. L., and Allen, E. V.: The Vascular Complications of Polycythemia. Am. Heart J. 13: 257, 1937.

3. Miller, H. R.: The Occurrence of Coronary Thrombosis in Polycythemia Vera. Am. J. Med. Sci. 198: 323-329, 1939.

4. Swartky, W. B., Weeder, S. D., and McLaughlin, E. F.: Thrombosis and Gangrene of Right Arm, associated with Polycythemia Vera: its Relation to "Effort Thrombosis". An. Surg. 116: 184, 1942.

5. Rosenthal, N., and Bassen, F. A.: Course of Polycythemia. Arch. Int. Med. 62: 903, 1938.

6. Dameshek, W., and Henstell, H. H.: The Diagnosis of Polycythemia. Ann. Int. Med. 13: 1360, 1940.

7. Tinney, W. S., Hall, B. E., and Giffin, H. Z.: Hematologic Complications of Polycythemia Vera. Proc. Staff Mtg. Mayo Clinic 18: 227, 1943.

8. Kirshbaum, J. D., and Preuss, F. S.: Leukemia A Clinical and Pathological Study of One Hundred and Twenty-three Fatal Cases in a Series of 400 Necropsies. Arch. Int. Med. 71: 777, 1943.

9. Rosenthal, N.: The Blood Picture in Purpura. J. Lab. and Clin. Med. 13: 303, 1928.

10. Estren, S., Medal, L. S., and Dameshek, W.: Pseudohemophilia. Blood 1: 504, 1946.

11. Forkner, C. E., Leukemia and Allied Disorders, Macmillan, N. Y., 1938.

12. LeSourd, L., and Pagniez, P.: La Retraction du Caillot Sanguin et Les Hematoblastes. J. de Physiol. et de Path. gen 9: 579, 1907.

13. Arthus, N., and Chapiro, T.,: Etudes Dur la Retraction du Caillot Sanguin. Arch. Internat. de Physiol. 6: 198, 1908.

14. Tocantins, L. M.: Platelets and the Spontaneous Syneresis of Blood Clots. Am. J. Physiol. 110: 278, 1934.

15. Tocantins, L. M.: Platelets and the Structure and Physical Properties of Blood Clots. Am. J. Physiol. 114: 709, 1936.

16. Hayem, G.: Du Caillot Non Retractile: Suppression de la Formation du Serum Sanguin Dans Quelques Etats Pathologique. Compt. Rend. Acad. Sci. 123: 894, 1896.

Bibliography (Continued)

17. Duke, W. W.: The Pathogenesis of Purpura Hemorrhagica with Special Reference to the Part Played by Blood Platelets. Arch. Int. Med. 10: 445, 1912.

18. Sooy, J. W., and Moise, T. S.: A Method for the Determination of the Coagulation Time and Retraction Time of the Blood. J. Lab. & Clin. Med. 12: 1113-15, 1927.

19. Almquist, A.: A Simple Method of Studying the Retraction of the Blood Clot. Acta Paediat. 20: 115, 1937.

20. Hirschboeck, J. S., and Coffey, W. L.: Method for Measuring Clot Retraction Time. Arch. Path. 33: 380, 1942.

21. Hirschboeck, J. S.: The Effect of Operation and Illness on Clot Retraction: Description of a New Method. J. Lab. and Clin. Med. 33: 347, 1948.

22. Boyce, F. F. and McFetridge, E. M.: A Serum Volume Test for the Hemorrhagic Diathesis in Jaundice. J. Lab. and Clin. Med. 23: 202, 1937.

23. Aggeler, P. M., Lucia, S.P., and Hamlin, L. M.: Blood Clot Retraction. 1. Measurement of the Extra Corpuscular Volume of the Clot. J. Lab. and Clin. Med. 28: 89, 1942.

24. Lucia, S. P., Aggeler, P. M., and Hamlin, L. H.: Blood Clot Retraction. II. The Significance of the Extracorpuscular Volume of the Clot and its Clinical Application. Am. J. of Med. Sci. 204: 507, 1942.

25. Lundsteen, E.: On the Clot in Retraction of the Blood. Acta Med. Scand. 112: 302, 1942.

26. Zahn, P.: Die Messung der Retraktion bei der Blutguinnung. Ann. Paediat. 163: 328, 1944.

27. Rosenthal, R. L., and Tobias, C. W.: Measurement of the Electric Resistance of Human Blood: Use in Coagulation Studies and Cell Volume Determinations. J. Lab. and Clin. Med. 33: 1110-1122, 1948.

28. Hirschboeck, J. S.:, Delayed Blood Coagulation and Absence of Clot Retraction in Collodion Lined Vessels. Proc. Soc. Exp. Biol. and Med. 45: 122, 1940.

29. Quick, A. J.: The Hemorrhagic Diseases, Charlces C. Thomas, Springield, Illinois, 1942.

30. Crafoord, C.: Preliminary Report on Post-Operative Treatment with Heparin as A Preventive of Thrombosis. Acta. Clin. Scand. 79: 407, 1937.

31. De Takats, G.: Heparin Tolerance: A Test of the Clotting Mechanism. Surg.Gyne, and Obst. 77: 31, 1943.

32. Waugh, T. R. and Ruddick, D. W.: A Test for Increased Coagulability of Blood. Can. M. A. J. 50: 547, 1944.

Bibliography (Continued)

33. Waugh, T. R. and Ruddick, D. W.: Studies on Increased Coagulability of Blood. Can. M. A. J. 51: 11, 1944.

34. Whittaker, J.: The Effect of Temperature on the Waugh and Ruddick Test for Increased Coagulation of Blood. Can. M. A. J. 52: 185, 1945.

35. Moses, C.: The Effect of Digitalis, Epinephrine and Surgery on the Response to Heparin. J. Lab. and Clin. Med. 30: 603, 1945.

36. Tuft, H. S. and Rosenfield, R. E.: Detection of Intra-vascular Clotting Tendency by Heparin Tolerance Principle. Am. J. Clin. Path. 17: 862, 1947.

37. Rosenbaum, E. E. and Barker, N. W.: A Test of the Coagulation Time of Blood Heparinized in Vitro; Studies of Normal Subjects and of Patients with Intravascular Thrombosis. J. Lab. and Clin. Med. 33: 1342, 1948.

38. Allen. J. G., Bogardus, G., Lacobson, L. O. and Spurr, C. L.: Some Observations on Bleeding Tendency in Thrombocytopenic Purpura. Ann. Int. Med. 27: 382, 1947.

39. Silverman, S.: A Modification of the Waugh-Ruddick Test for Increased Coagulability of the Blood, and Its Application to the Study of Postoperative Cases. Blood 3: 147, 1948.

40. Hagedorn, A. B. and Barker, N. W.: Coagulation Time of Blood Heparinized in Vitro: Correlation of Results With Those of the Heparin Tolerance Test. J. Lab. and Clin. Med. 32: 1087, 1947.

41. Jaques, L. B., and Ricker, A. G.: The Relationship Between Heparin Dosage and Clotting Time: Blood 3: 1197, 1948.

42. De Takats, G., and Fowler, E. F.: The Problem of Thrombo-embolism. Surg. 17: 153, 1945.

43. Ogura, J. H., Fetter, N. R., Blankenhorn, M. A., and Glueck, H. I.: Changes in Blood Coagulation Following Coronary Thrombosis, Measured by the Heparin Retarded Clotting Test (Waugh and Ruddick Test). J. Clin. Invest. 25: 586, 1946.

44. Hagedorn, A. and Barker, N. W.: Response of Persons With and Without Intravascular Thrombosis to a Heparin Tolerance Test. Am. J. Heart J. 35: 603, 1948.

45. Hilton, J. H. B., Cameron, W. M., Mills, E. S., and Townsend, S. R.: The Questionable Importance of Blood Changes in Coronary Occlusion. Can. M. A. J. 59: 447, 1948.

46. Best, C., and Jaques, L.: Heparin in Blood Clotting and Thrombosis. Ann. N. Y. Acad. Sci. 49: 501, 1948.

47. Rosenfield, R. E., and Tuft, H. W.: Estimation of Prothrombin Level From Prothrombin Time. Am. J. Clin. Path. 17: 5, 1947.

48. Morrison, I. R.: Qualitative Changes in Fibrinogen which Influence the Erythrocyte Sedimentation Rate and the Clot Retraction Time. Am. J. Med. Sci. 211: 325, 1946.

Bibliography (Continued)

49. Minot, G. R., and Buckman, R. E.: The Blood Platelets in Leukemias. Am. J. Med. Sci. 169: 477, 1925.

50. Allen, J. G., Sanderson, M., Milham, M., Kirschon, A., and Jacobson, L. O. Heparinemia (?). An Anticoagulant in the Blood of Dogs with Hemorrhagic Tendency After Total Body Exposure to Roentgen Rays. J. Exp. Med. 87:71, 1948.

51. Smith, T. R., Jacobson, L. O., Spurr, C. L., Allen, J. G., and Block, M. H. A Coagulation Defect Produced by Nitrogen Mustard. Science 107: 474, 1948.

52. Holobeck, J. E., Hendrick, J. V., and Hollis, W. J.: Toluidine Blue in Bleeding Associated with Thrombopenia. J. A. M. A. 139: 214, 1949.

53. Harrop, G. A., and Wintrobe, M. M.: Polycythemia, in Handbook of Hematology, Hoeber, N. Y., 1938.

54. Doré, G. R., Delisconet, R., and Callegari.: Trois Cas D'érythremie: Variabilité en Chiffre Globulaire: Constance des Troubles de la Coagulabilité. Bull. et Mem. Soc. Med. D. Hôp. de Paris. 53: 1287, 1937.

55. Guilford, J. P.: Fundamental Statistics in Psychology and Education, McGraw-Hill, N. Y., 1942.

56. Aggeler, P. M., Howard, J., and Lucia, S. P.: Platelet Counts and Platelet Function. Blood 1: 472, 1946.

57. Schweppe, J., Lindberg, H. A., and Barker, M.: Experience with hree Vascular Fragility Tests in Hypertension. Am Heart J. 35: 393, 1948.

58. Nygaard, K.: Hemorrhagic Diseases, C. V. Mosby, St. Louis, 1941.

59. Quick, A. J., Honorato, R., and Stefanini, M.: The Value and the Limitations of the Coagulation Time in the Study of the Hemorrhagic Diseases. Blood 3: 1120, 1948.

60. Sharp, E. A., Wolter, J. G., and Wonder Heide, E. C.: Prothrombin in Disorders of the Blood. Am. J. Clin. Path. 14: 44, 1944.

61. Jorpes, J. E.: Heparin in the Treatment of Thrombosis. Oxford, London, 1946, 2nd ed.

62. Jorpes, J. E.: Origin and Physiology: The Specific Rx in Thrombosis. Ann. Int. Med. 27: 361, 1947.

63. Wright, I., and Foley, W.: Use of Anticoagulants in the Treatment of Heart Disease. Am. J. Med. 3: 718, 1947.

64. Loewe, L., and Hirsch, E.: Heparin in the Treatment of Thromboembolic Disease. J. A. M. A. 133: 1263, 1947.

65. Allen, A. W., Linton, R. R., and Donaldson, G. A: Venous Thrombosis and Pulmonary Embolism. J. A. M. A. 133: 1268, 1947.

Bibliography (Continued)

66. Peters, H., Doenges, J., and Brambel, C.: Further Experiences with Dicumerol Therapy in Coronary Thrombosis. So. Med. J. 41: 526, 1948.

67. Howell, W. H.: The Purification of Heparin and its Chemical and Physiological Reactions. Bull. Johns Hopkins Hosp. 42: 199, 1928.

68. Quick, A. J.: On the Action of Heparin and its Relation to Thromboplastin. Am. J. Phys. 115: 317, 1936.

69. Brinkhous, K. M., Smith, H. P., Warner, E. D., and Seegers, W. H.: An Unidentified Substance which Acts in Conjunction with Heparin to Prevent Conversion of Prothrombin into Thrombin. Am. J. Physiol. 125: 683, 1939.

70. Ferguson, J. H.: The Action of Heparin, Serum Albumin (Crystalline) and Salmine on Blood Clotting Mechanisms (in vitro). Am. J. Phyiol. 130:759, 1940.

71. Ziff, M., and Chargoff, E.: Mode of Action of Heparin. J. Biol. Chem. 136: 689, 1940.

72. Jaques, L. B., and Ramustard.: Some Factors Influencing the Anticoagulant Action of Heparin. Bioch. J. 34: 153, 1940.

73. Wilander, O.: Studien Uber Heparin. Skand. Arch. f. Physiol. 81: 15, 1938.

74. Oliver, J., Bloom, F., and Mangieri, C.: On the Origin of Heparin. J. Exp. Med. 86: 107, 1947.

75. Tocantins, L. M.: Heparinemia. Blood 3: 1304, 1948.

76. Howell, W. H.: The Purification of Heparin and Its Presence in Blood. Am. J. Physiol. 71: 55w, 1925.

77. Quick, A. J.: On the Coagulation Defect in Peptone Shock. A Consideration of Antithrombins. Am. J. Physiol. 116: 535, 1936.

78. Waters, E. T., Markowitz, J., and Jaques, L. B.: Anaphylaxis in the Liverless Dog and Observations on the Anticoagulant of Anaphylactic Shock. Science 87: 582, 1938.

79. Jaques, L. B., and Waters, E. T.: The Identity and Origin of the Anticoagulant of Anaphylactic Shock in the Dog. J. Physiol. 99: 454, 1941.

80. Eagle, H., Johnston, C. G., and Ravdin, I. S.: On the Prolonged Coagulation Time Subsequent to Anaphylactic Shock. Bull. Johns Hopkins Hosp. 60: 428, 1937.

81. Volkert, M.: Antithrombin Content of the Blood in Peptone Intoxication of Rabbits. Acta. Physiol. Scand. 5: 365, 1943.

82. Rosenthal, R. L.: Coagulation Studies in the Rabbit After Total Body X-irradiation. (To be published).

83. Kohn, H. I., Robinett, P. W., and Cupp, M. N.: The Effect of Rutin upon the Response of the Rat to total Body X-irradiation. AECD 2176, 1948.

Bibliography (Continued)

84. Quick, A. J., Shanberge, J. N., and Stefanini, M.: The Effect of Heparin on Platelets in Vivo. J. Lab. and Clin. Med. 33: 14241, 1948.

85. Rosenthal, R. L.: Studies on the Effect of Total Body X-irradiation on Mast Cells and Coagulation in Mice. (To be published.)

86. Jaques, L. B., Waters, E. T., Charles, A. F.: A Comparison of the Heparins of Various Mammalian Species. J. Biol. Chem. 144: 229, 1942.

87. Chargoff, E., and Olson, K. B.: Studies on the Chemistry of Blood. VI. Studies on the Action of Heparin and Other Anticoagulants. The Influence of Protamine on the Anticoagulant Effect in Vivo. J. Biol. Chem. 122: 153, 1937.

88. Chargoff, E.: Studies on the Chemistry of Blood Coagulation. VII. Protamines and Blood Clotting. J. Biol. Chem. 125: 671, 1938.

89. Jaques, L. B.: The Reaction of Heparin with Proteins and Complex Bases. Biochem. J. 37: 189, 1943.

90. Jaques, L. B., Mitford, B., and Ricker, A. G.: The Metachromatic Activity of Heparin. Rev. Can. Biol. 6: 740, 1947.

91. Mackowiak, E. J., and Barnard, R. D.: Comparative in Vitro Neutralization of Heparin. J. Am. Parm. Ass. 36: 383, 1947.

92. Quick, A. J., Ota, R. K., and Barnofsky, I. D.: On the Thrombopenia of Anaphylactic and Peptone Shock. Am. J. Physiol. 145: 273, 1946.

93. Kinsell, L. W., Kopeloff, L. M., Zwemel, R. L., and Kopeloff, N.: Blood Constituents during Anaphylactic Shock in the Monkey. J. Immunol. 42: 35, 1941.

94. Jacobson, L. O., Marks, E. K., Gaston, E., Allen, J. G., and Block, M. H.: The Effect of Nitrogen Mustard and X-irradiation on Blood Coagulation. J. Lab. and Clin. Med. 33: 1566, 1948.

95. Copley, A. L. and Robb, T. P.: Studies on Platelets. II. The Effect of Heparin on the Platelet Count in Vitro. Am. J. Clin. Path. 12: 416, 1942.

96. Copley, A. L. and Robb, T. P.: Studies on Platelets III. The Effect of Heparin in Vivo on the Platelet Count in Mice and Dogs. Am. J. Clin. Path. 12: 563, 1942.

97. Copley, A. L.: Hemorrhagic Diathesis in Hiroshima, Nagasaki and at Bikini Atomic Bomb Test. J. A. M. A. 136: 145, 1948.

98. Fidlar, E., and Jaques, L. B.: The Effect of Commercial Heparin on the Platelet Count. J. Lab. and Clin. Med. 33: 1410, 1948.

99. Rosenthal, N.: Blood Platelets and Megakaryocytes, Handbook of Hematology, Hoeber, N. Y., 1938.

100. Quick, A. J., and Stefanini: Activation of Plasma Thromboplastinogen and Evidence of an Inhibitor. Proc. Soc. Exp. Biol. Med. 67: 111, 1948.

Bibliography (Continued)

101. Ware, A. G., Fahey, J. L., and Seegers, W. H.: Platelet Extracts, Fibrin Formation and Interaction of Purified Prothrombin and Thromboplastin. Am. J. Physiol. 154: 140, 1948.

102. Wright, H. P.: Changes in the Adhesiveness of Blood Platelets Following Parturition and Surgical Operations. J. Path. and Bact. 54: 461, 1942.

103. Morrison, M., Richter, I. H., and Loewe, L.: Increased Blood Platelet Clumping in Thromboembolic Disease. Am. J. Clin. Path. 18: 879, 1948.

104. Weiner, M., Zeltmacher, K., Reich, C. and Shapiro, S.: Platelet Adhesiveness. Blood 3: 1275, 1948.

## CAPTIONS FOR FIGURES

Figure 1. Typical electric resistance curve of clot retraction. The various features of the curve are explained. The diagrams represent the appearance of the blood in the electric resistance tube at the various stages of clotting and retraction.

Figure 2. Upper left and right: The average electric resistance curve for 52 normal subjects and the 90 percent range for 40 minutes and 4 hours. After the first 40 minutes, a very wide spread of the 90 percent range is apparent. Lower left and right: Clot retraction curves for aliquot samples of normal blood using a glass tube and a collodion-lined tube. No clot retraction is seen by inspection in collodion-lined tubes. The point between 25 and 30 minutes at which the curve for the collodion-lined tube stops rising corresponds to the time of separation of the retracting clot from the vessel wall in the glass tube. The resistance changes prior to this point are produced by internal retraction which occurs in similar manner in both the glass and collodion-lined tubes.

Figure 3. Distribution of the clot retraction rates for normal subjects and certain groups of patients. Shows the increased clot retraction rates for 12 polycythemic patients, while 11 leukemia patients show decreases. A difference between the values for a small group of patients without primary hematopoietic disease and those with hematopoietic disease or lymphomas is apparent.

Figure 4. Average electric resistance curves for certain groups of subjects as indicated. Slopes of the broken lines, drawn to connect the 10 and 30 minute values, represent the clot retraction rates. The close agreement in clot retraction rate among the normal subjects and patients with leukemia and polycythemia with platelets under 400,000 is apparent. The initial values, closely correlated with the hematocrit, show marked differences for these groups. Polycythemia subjects with platelets over 400,000 show a very high clot retraction rate.

Figure 5. Results of the multiple tube heparin clotting test for 20 normal subjects, plotted on semi-logarithmic scales. There is a straight line or logarithmic relation between the clotting time and heparin concentrations from .004 to .012 mg. per cc. of blood. Values for the first two tubes (0 and .002 mg. of heparin) fall below an extrapolation (broken line) of this straight line. This is explained by the incubation period of heparin in the blood, during which time the anticoagulant effect of heparin is potentiated or increased.

Figure 6. Results of the multiple tube heparin clotting test for normal subjects and patients with polycythemia and leukemia. Marked increases above normal are shown by many leukemic patients, while most of the patients with polycythemia are within the normal range. Tube 3 (.004 mg heparin per 1 cc. blood), selected as the heparin concentration used for the single tube, simplified test, is seen to give as adequate information as any of the other tubes, and to have the most convenient clotting time range for routine use.

Captions for Figures (Continued)

Figure 7. Distribution of the clotting times for normal subjects and patients as indicated. All results of this test were in the normal range. This is indicative of the limited value of this widely used test of coagulation.

Figure 8. Distribution of the heparin clotting times. Shows a well defined range of normal subjects and abnormalities of certain groups of patients, particularly, the increase shown by half of the leukemia patients.

Figure 9. Representative clot retraction or electric resistance curves for various patients. Some pertinent data are as follows: a. C.S., clot retraction rate 4.7, platelet count 166,000 and heparin clotting time 118 min. b. E.W., clot retraction rate 8.6, platelets 520,000 and hep. cl. time 27 min. c. M.R., cl. ret. rate 3.7, platelets 200,000. d. G.S., cl. ret. rate 10.2, platelets 200,000 and hep. cl. time 26 min. e. P.S., cl. ret. rate 4.9, platelets 254,000 and hep. cl. time 37 minutes. f. E.W., cl. ret. rate 2.4, platelets 83,000 and hep. cl. time 35 min.

Figure 10. Atypical electrical resistance curve. Explanation of the features of this type of curve, which was often seen in leukemia patients with very rapid red cell sedimentation rates, low hematocrits and platelets, is given. Data for the patients are: M.M., clot retraction rate 2.4 ohm-cm. per min., platelet count 160,000, hematocrit 29 percent and heparin clotting time 58 min. R.J., cl. ret. rate 0.8, platelets 132,000, hematocrit 20 percent, heparin clotting time 122 min. and bleeding time over 20 min. J.H., other findings are shown in Figure 11.

Figure 11. Changes in coagulation and hematological tests over a period of several months in a patient with chronic lymphatic leukemia. During this period patient received the following treatment: 6 millicuries $P^{32}$, 2.3 mc. $Y^{90}$, 1950 Roentgens (r) of x-ray in doses of 100 to 150 r to lymph nodes and tranfusions. The coagulation tests, particulary the heparin clotting time, are seen to show marked improvement as did the clinical and hematological condition.

Figure 12. Representative clot retraction curves for 4 patients with polycythemia vera. Coagulation findings are listed in the following order, clot retraction rate in ohm-cm. per minute, platelet count and heparin clotting time in minutes: a. F.T., 17.6, 580,000, and 24. b. E.B., 13.9, 580,000 and 20. c. L.A., 8.2, 320,000 and 29. d. B.L., 4.4, 190,000 and 58.

Figure 13. Heparin clotting time (multiple tubes performed on M.A., polycythemia vera, on 4 different occasions as the hematocrit and platelet count changed as shown in response to treatment with 11 millicures of $Y^{90}$ and 5 500 cc. phlebotomies during the period of observation. At left, plot of clotting times versus heparin concentration in mg. per cc. <u>of blood</u> gives 4 separate curves. At right, plot of clotting times versus heparin concentration in mg. per cc. <u>of plasma</u> shows all points falling near a single curve. This shows the change in heparin clotting time to be related to the hematocrit change.

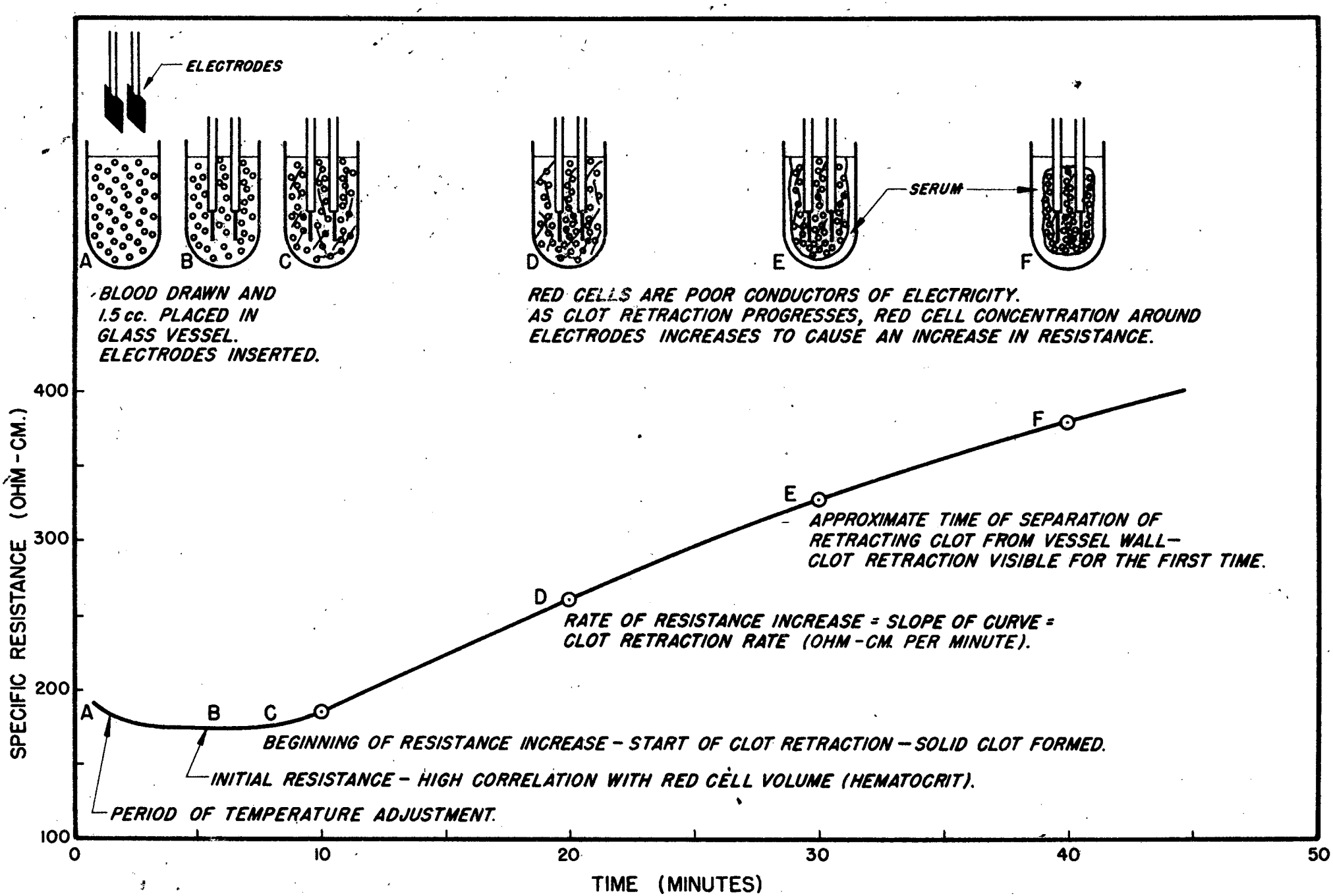
ELECTRODES
A
B
C
D
E
F
SERUM
BLOOD DRAWN AND
1.5 cc. PLACED IN
GLASS VESSEL.
ELECTRODES INSERTED.
RED CELLS ARE POOR CONDUCTORS OF ELECTRICITY.
AS CLOT RETRACTION PROGRESSES, RED CELL CONCENTRATION AROUND
ELECTRODES INCREASES TO CAUSE AN INCREASE IN RESISTANCE.
APPROXIMATE TIME OF SEPARATION OF
RETRACTING CLOT FROM VESSEL WALL—
CLOT RETRACTION VISIBLE FOR THE FIRST TIME.
RATE OF RESISTANCE INCREASE = SLOPE OF CURVE =
CLOT RETRACTION RATE (OHM-CM. PER MINUTE).
BEGINNING OF RESISTANCE INCREASE – START OF CLOT RETRACTION – SOLID CLOT FORMED.
INITIAL RESISTANCE – HIGH CORRELATION WITH RED CELL VOLUME (HEMATOCRIT).
PERIOD OF TEMPERATURE ADJUSTMENT.
SPECIFIC RESISTANCE (OHM-CM.)
400
300
200
100
0
10
20
30
40
50
TIME (MINUTES)

FIGURE 1

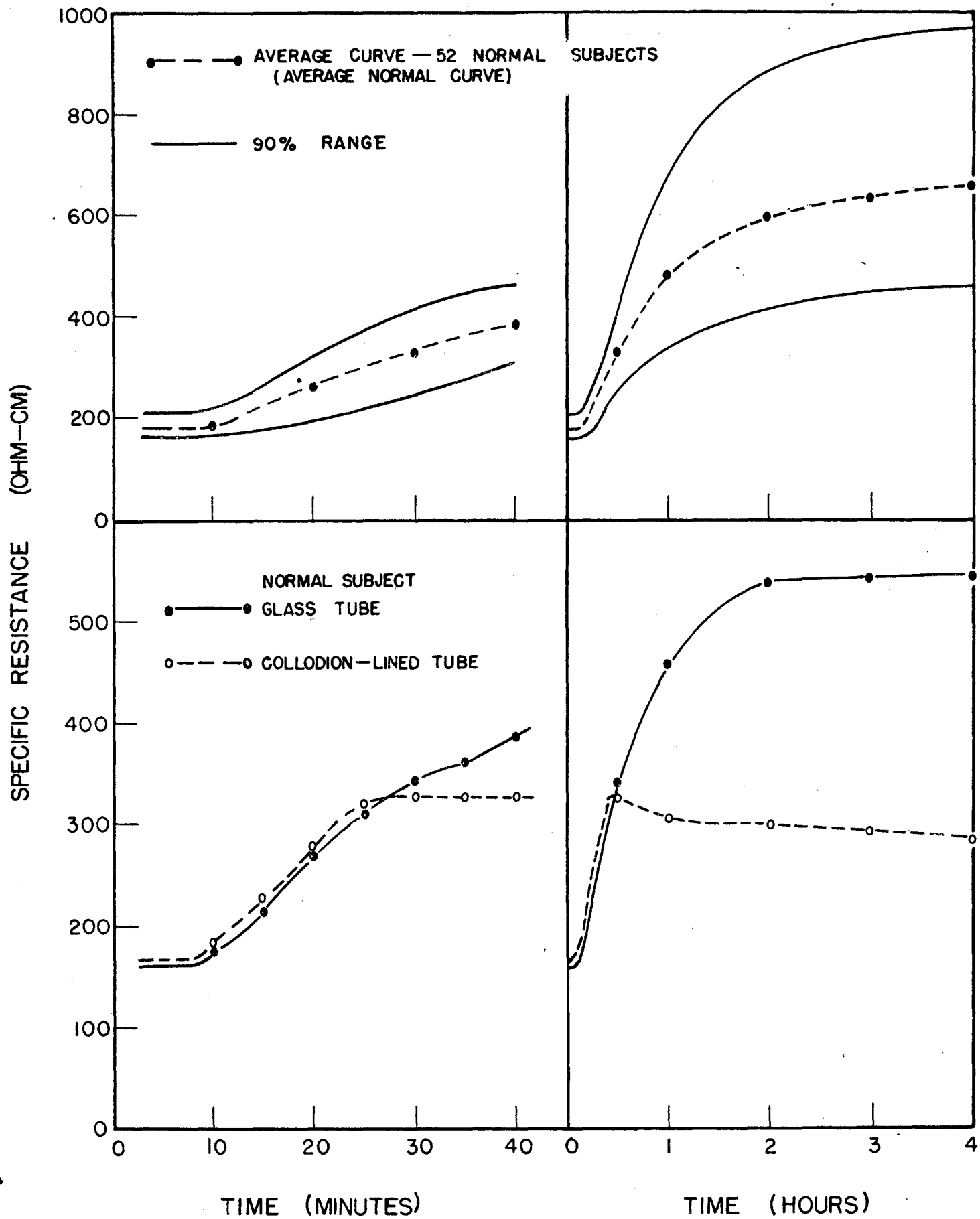
AVERAGE CURVE — 52 NORMAL SUBJECTS
(AVERAGE NORMAL CURVE)
90% RANGE
NORMAL SUBJECT
GLASS TUBE
COLLODION—LINED TUBE
SPECIFIC RESISTANCE (OHM—CM)
1000
800
600
400
200
0
500
400
300
200
100
0
0
10
20
30
40
0
1
2
3
4
TIME (MINUTES)
TIME (HOURS)

FIGURE 2

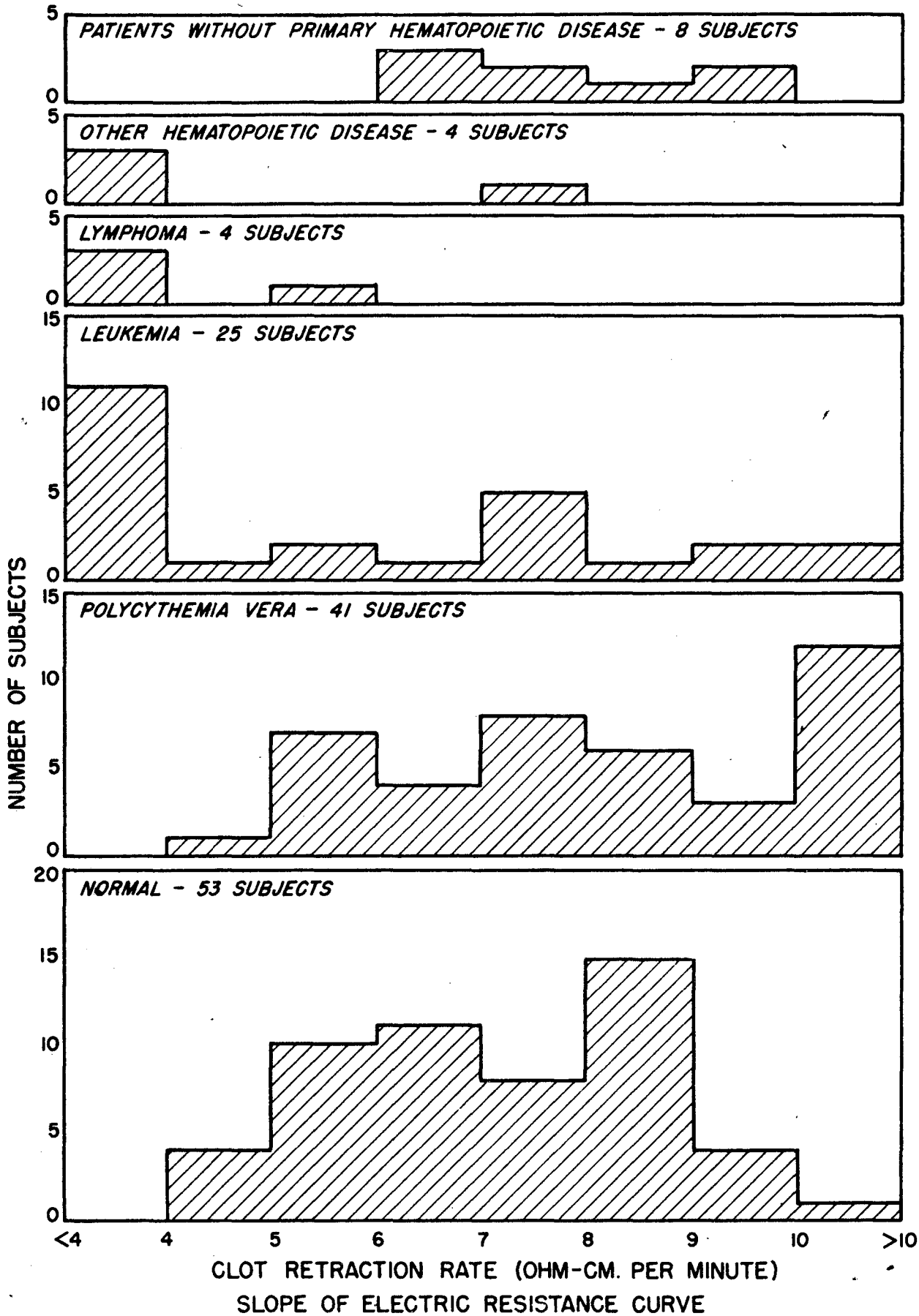
PATIENTS WITHOUT PRIMARY HEMATOPOIETIC DISEASE - 8 SUBJECTS
OTHER HEMATOPOIETIC DISEASE - 4 SUBJECTS
LYMPHOMA - 4 SUBJECTS
LEUKEMIA - 25 SUBJECTS
POLYCYTHEMIA VERA - 41 SUBJECTS
NORMAL - 53 SUBJECTS
NUMBER OF SUBJECTS
<4
4
5
6
7
8
9
10
>10
CLOT RETRACTION RATE (OHM-CM. PER MINUTE)
SLOPE OF ELECTRIC RESISTANCE CURVE

FIGURE 3

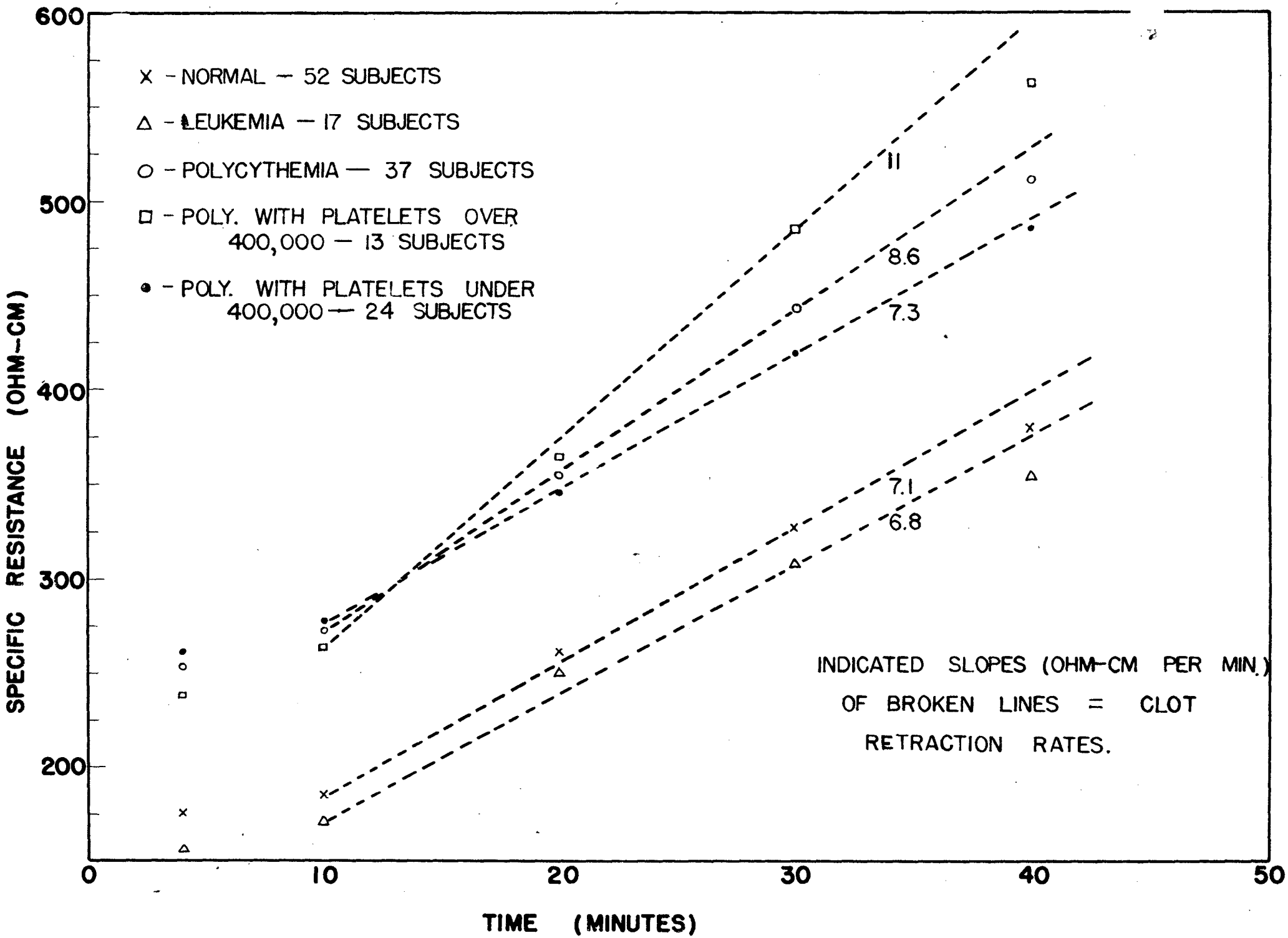
X - NORMAL — 52 SUBJECTS
△ - LEUKEMIA — 17 SUBJECTS
○ - POLYCYTHEMIA — 37 SUBJECTS
□ - POLY. WITH PLATELETS OVER 400,000 — 13 SUBJECTS
● - POLY. WITH PLATELETS UNDER 400,000 — 24 SUBJECTS
11
8.6
7.3
7.1
6.8
INDICATED SLOPES (OHM-CM PER MIN.) OF BROKEN LINES = CLOT RETRACTION RATES.
SPECIFIC RESISTANCE (OHM-CM)
600
500
400
300
200
0
10
20
30
40
50
TIME (MINUTES)

FIGURE 4

4F1721

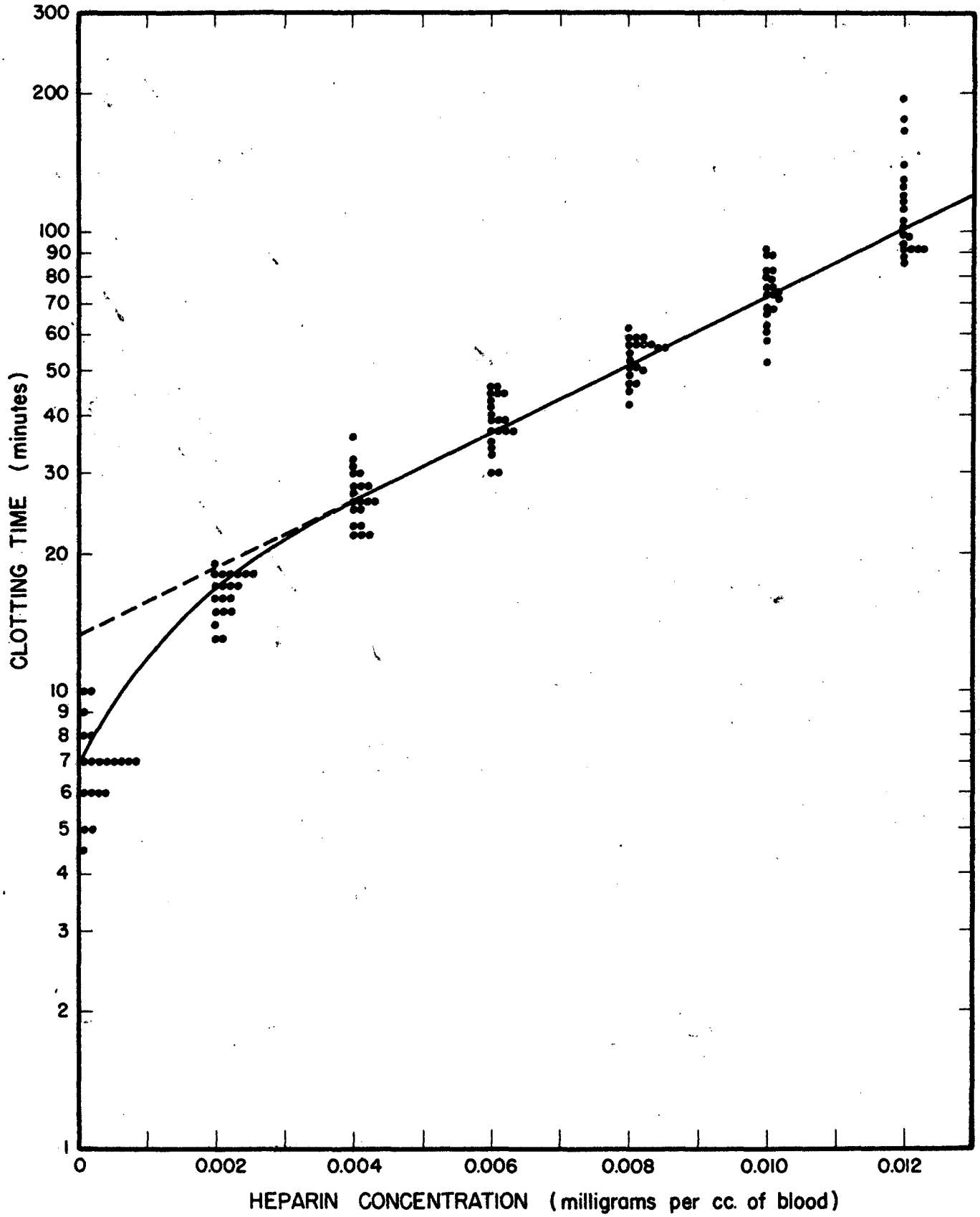


FIGURE 5

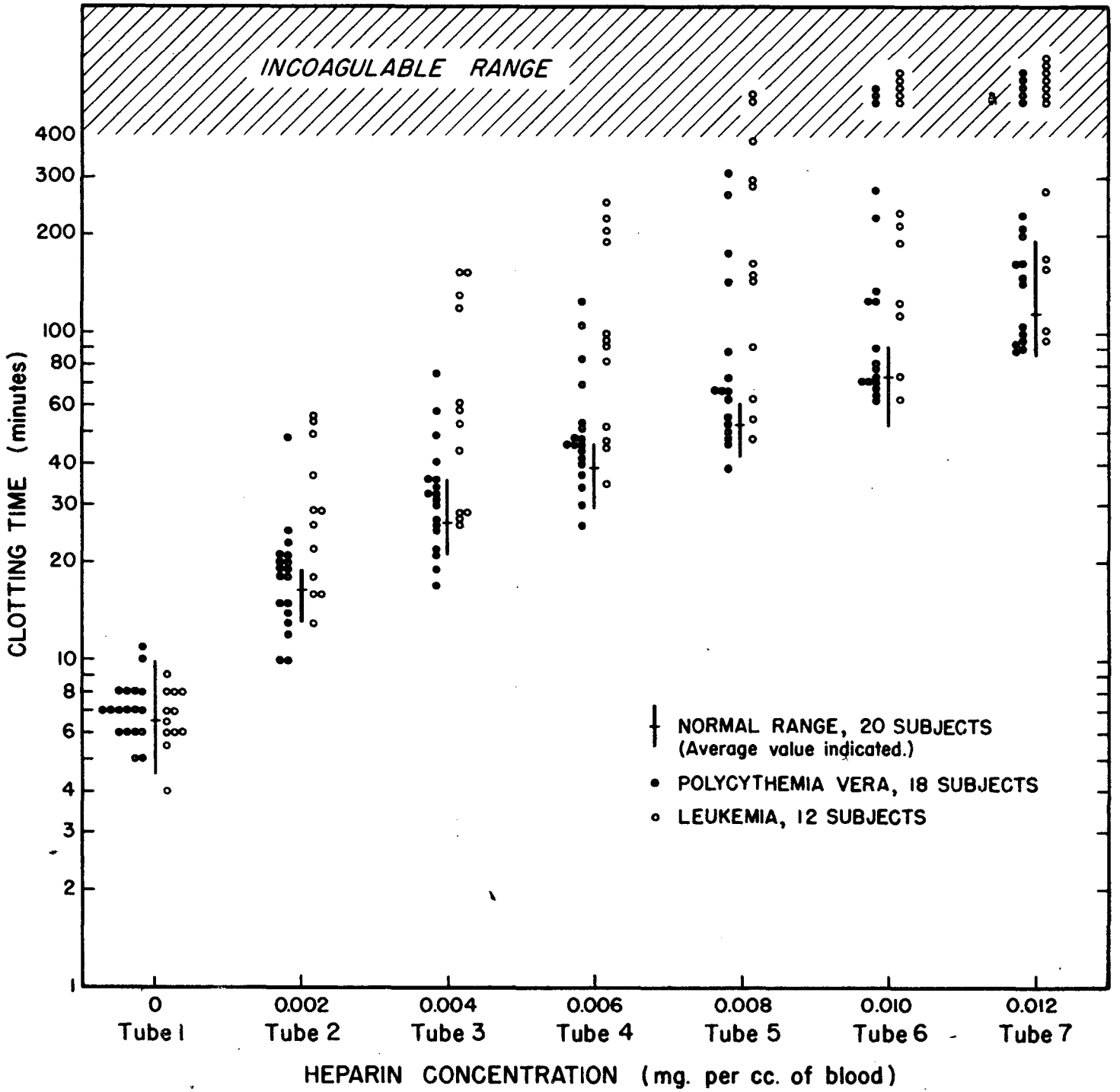
INCOAGULABLE RANGE
CLOTTING TIME (minutes)
400
300
200
100
80
60
40
30
20
10
8
6
4
3
2
1
NORMAL RANGE, 20 SUBJECTS
(Average value indicated.)
POLYCYTHEMIA VERA, 18 SUBJECTS
LEUKEMIA, 12 SUBJECTS
0
Tube 1
0.002
Tube 2
0.004
Tube 3
0.006
Tube 4
0.008
Tube 5
0.010
Tube 6
0.012
Tube 7
HEPARIN CONCENTRATION (mg. per cc. of blood)

FIGURE 6

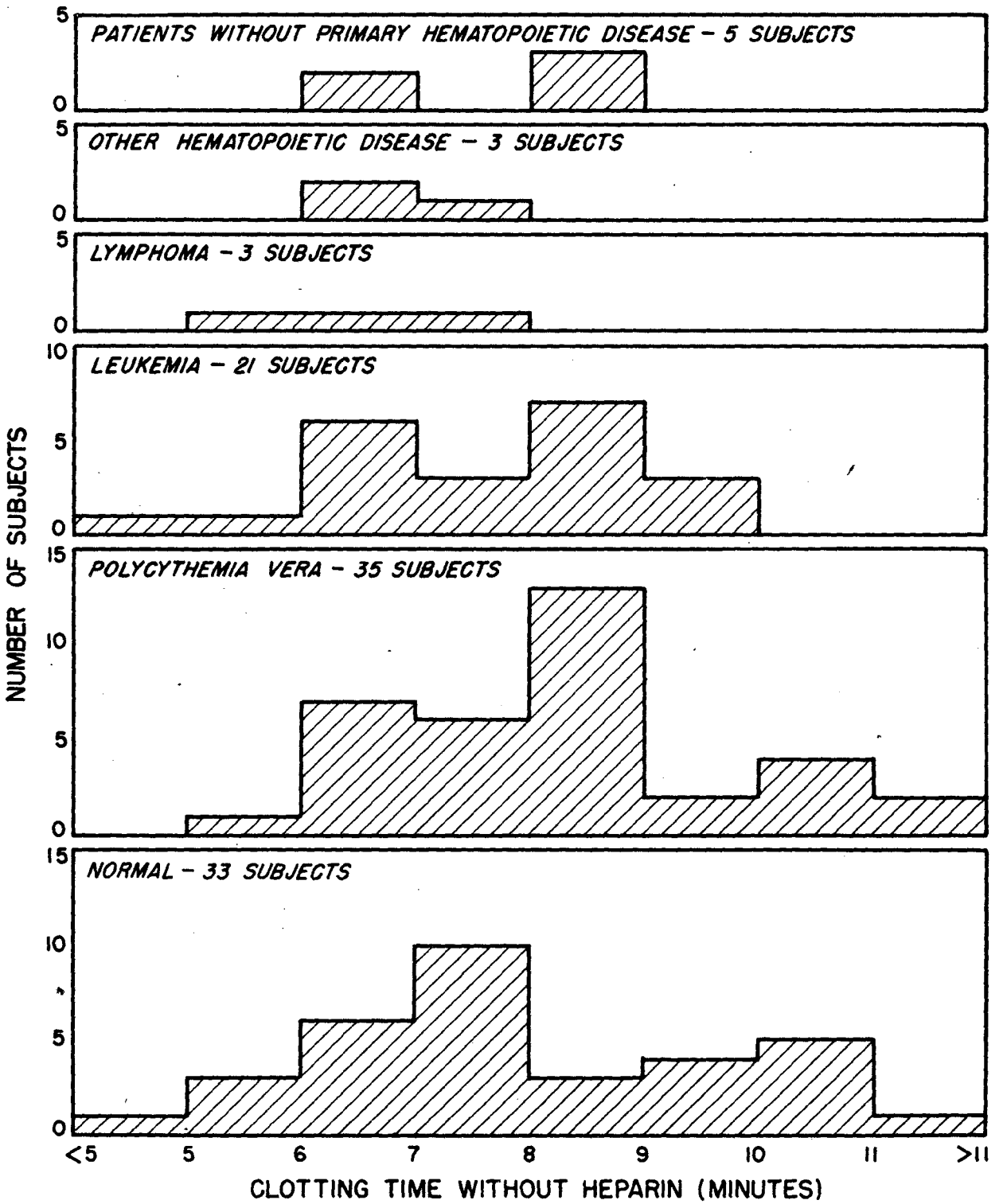
PATIENTS WITHOUT PRIMARY HEMATOPOIETIC DISEASE – 5 SUBJECTS
OTHER HEMATOPOIETIC DISEASE – 3 SUBJECTS
LYMPHOMA – 3 SUBJECTS
LEUKEMIA – 21 SUBJECTS
POLYCYTHEMIA VERA – 35 SUBJECTS
NORMAL – 33 SUBJECTS
NUMBER OF SUBJECTS
0
5
10
15
<5
5
6
7
8
9
10
11
>11
CLOTTING TIME WITHOUT HEPARIN (MINUTES)

FIGURE 7

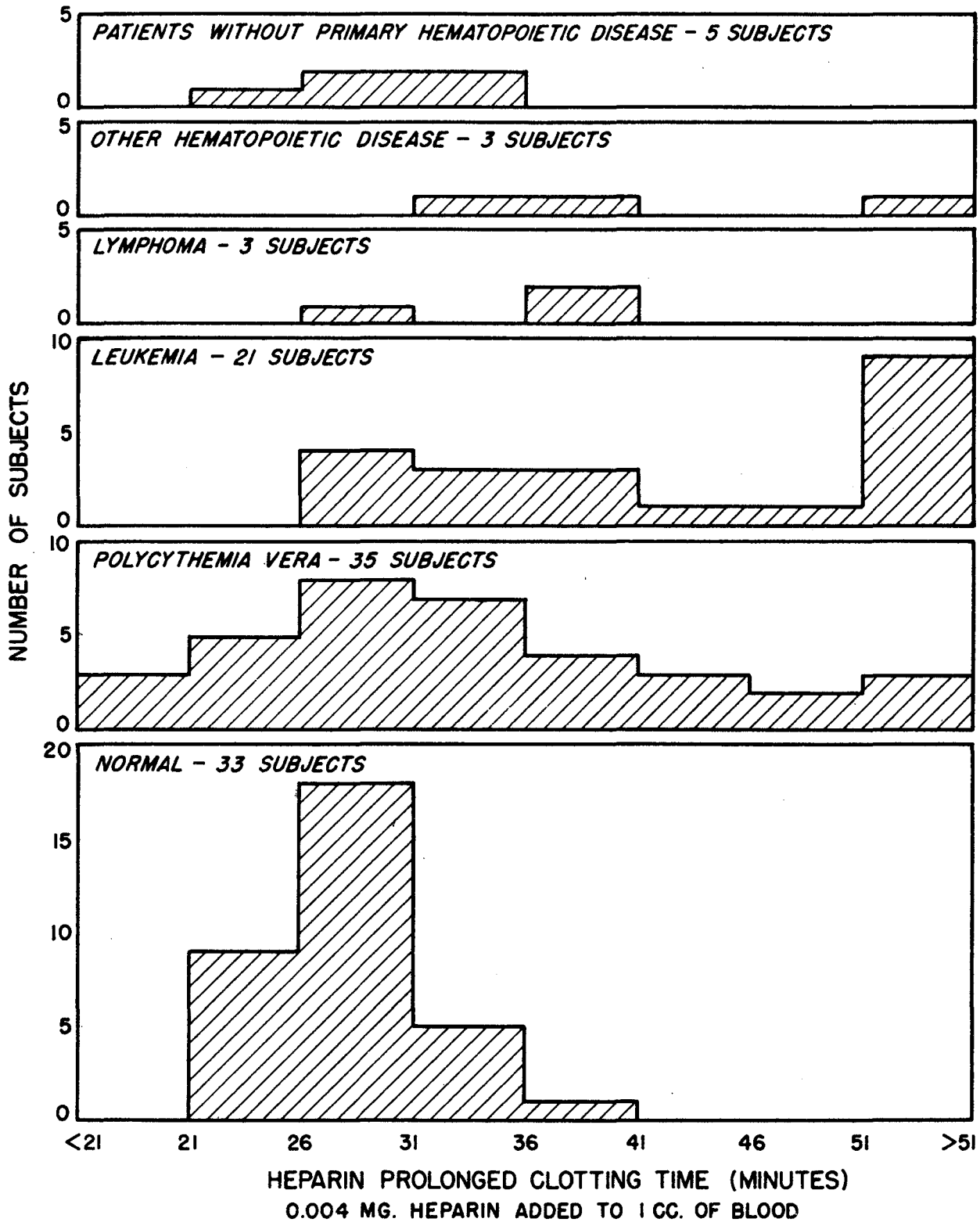
PATIENTS WITHOUT PRIMARY HEMATOPOIETIC DISEASE - 5 SUBJECTS
OTHER HEMATOPOIETIC DISEASE - 3 SUBJECTS
LYMPHOMA - 3 SUBJECTS
LEUKEMIA - 21 SUBJECTS
POLYCYTHEMIA VERA - 35 SUBJECTS
NORMAL - 33 SUBJECTS
NUMBER OF SUBJECTS
<21
21
26
31
36
41
46
51
>51
HEPARIN PROLONGED CLOTTING TIME (MINUTES)
0.004 MG. HEPARIN ADDED TO 1 CC. OF BLOOD

FIGURE 8

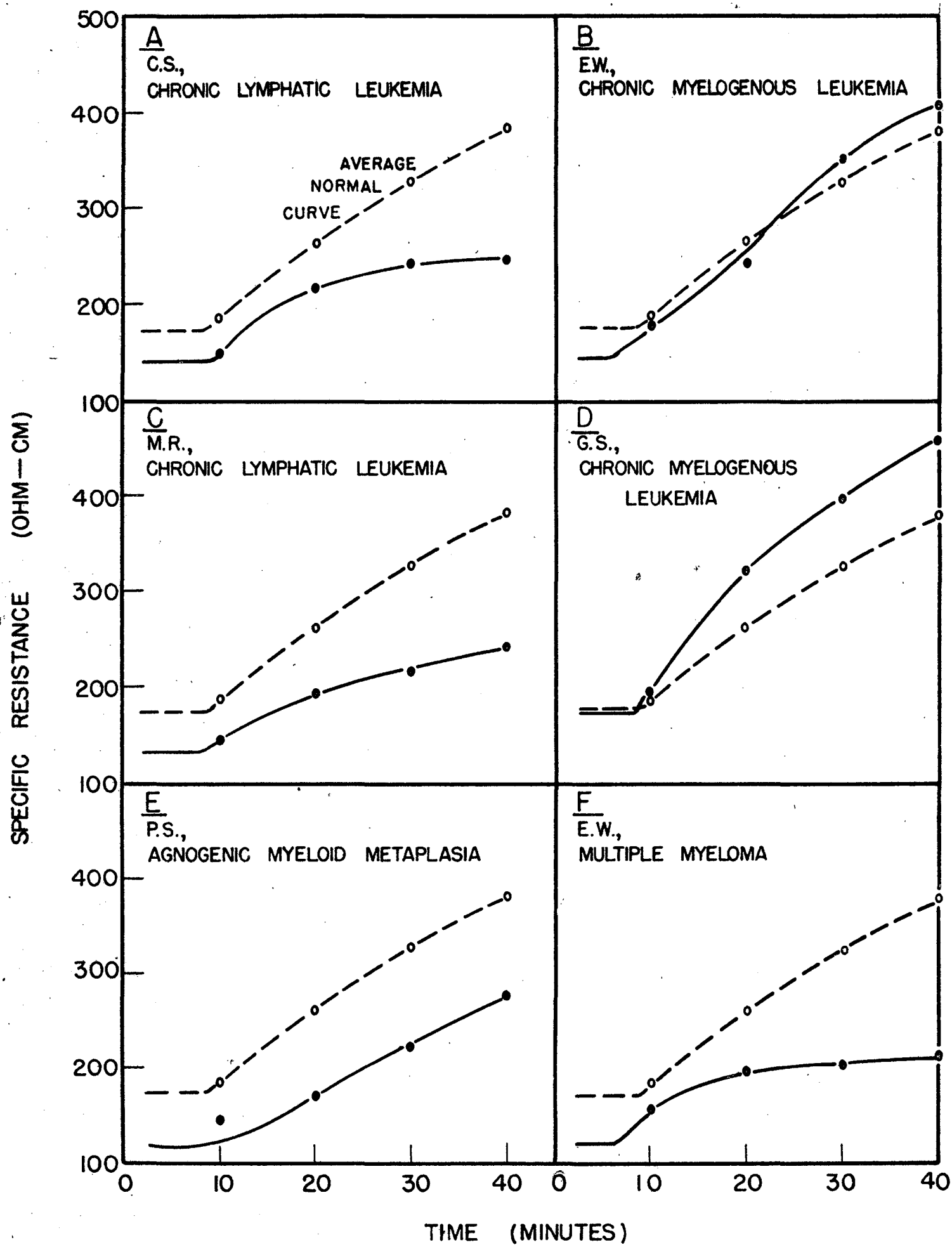
A
C.S.,
CHRONIC LYMPHATIC LEUKEMIA
AVERAGE
NORMAL
CURVE
B
E.W.,
CHRONIC MYELOGENOUS LEUKEMIA
C
M.R.,
CHRONIC LYMPHATIC LEUKEMIA
D
G.S.,
CHRONIC MYELOGENOUS
LEUKEMIA
E
P.S.,
AGNOGENIC MYELOID METAPLASIA
F
E.W.,
MULTIPLE MYELOMA
SPECIFIC RESISTANCE (OHM—CM)
500
400
300
200
100
0
10
20
30
40
TIME (MINUTES)

FIGURE 9

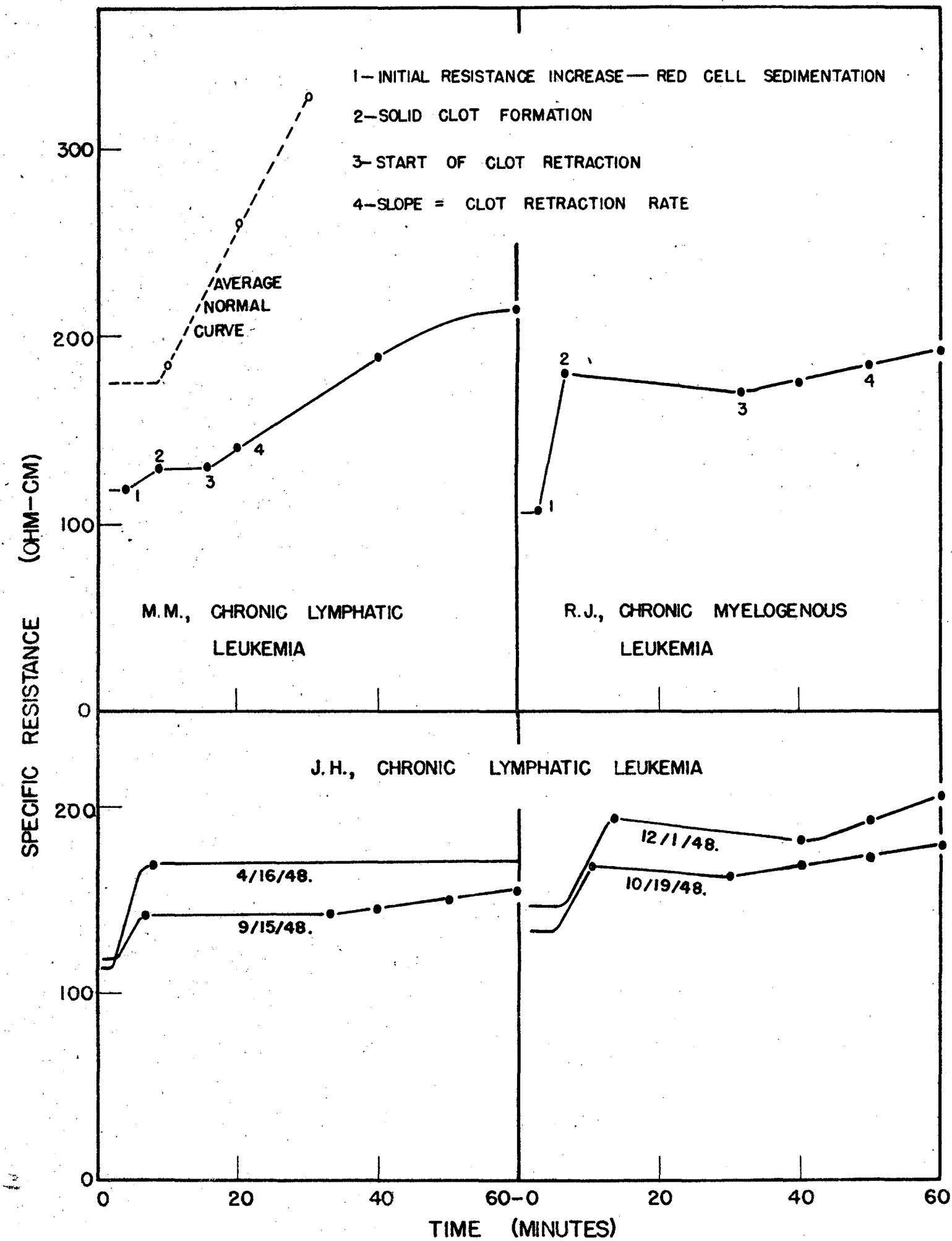
1—INITIAL RESISTANCE INCREASE—RED CELL SEDIMENTATION
2—SOLID CLOT FORMATION
3—START OF CLOT RETRACTION
4—SLOPE = CLOT RETRACTION RATE
AVERAGE NORMAL CURVE
M.M., CHRONIC LYMPHATIC LEUKEMIA
R.J., CHRONIC MYELOGENOUS LEUKEMIA
J.H., CHRONIC LYMPHATIC LEUKEMIA
4/16/48.
9/15/48.
12/1/48.
10/19/48.
SPECIFIC RESISTANCE (OHM-CM)
TIME (MINUTES)
0
100
200
300
20
40
60-0
60

FIGURE 10

4F2381

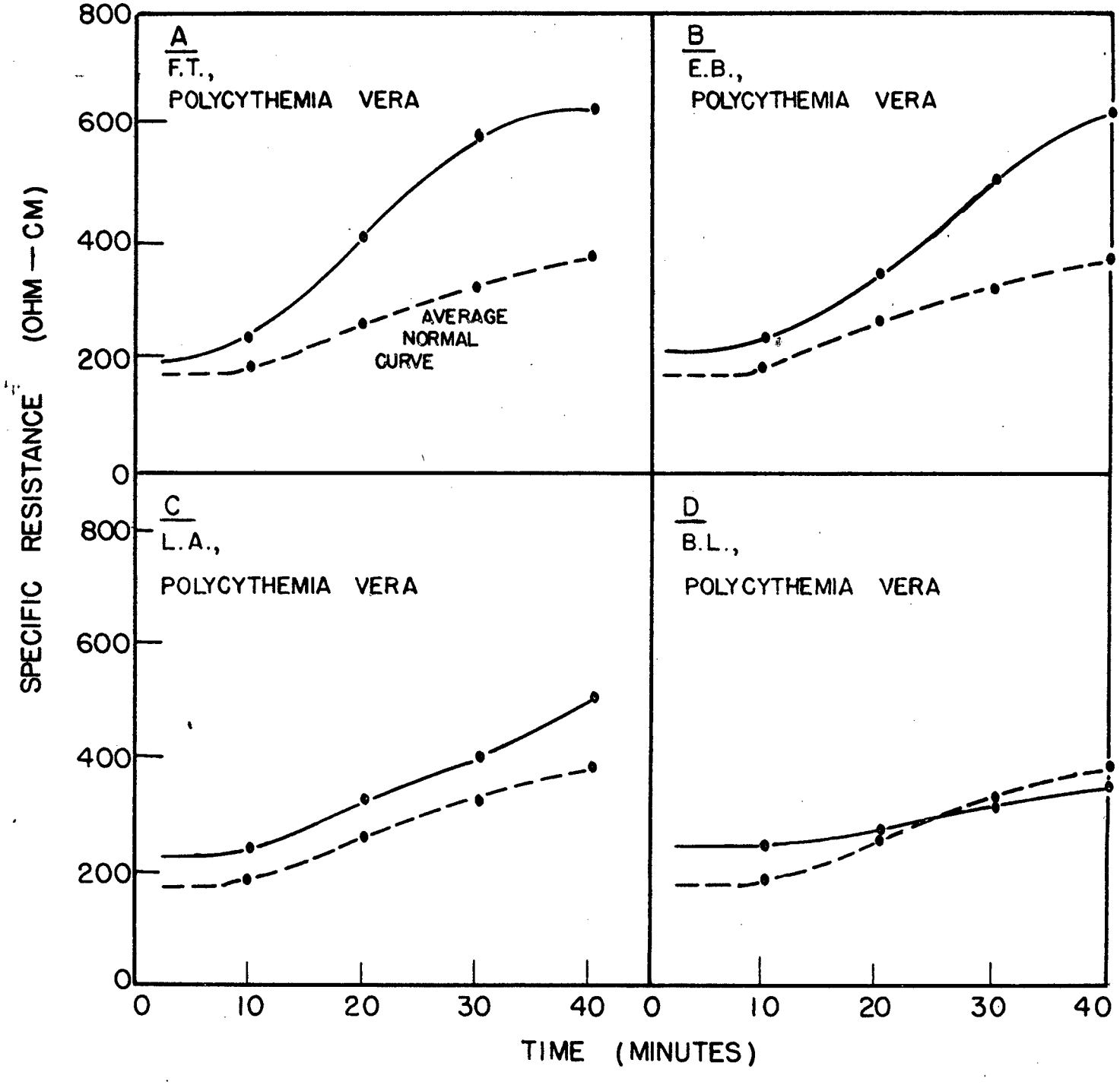


FIGURE 11

J. H. CHRONIC LYMPHATIC LEUKEMIA

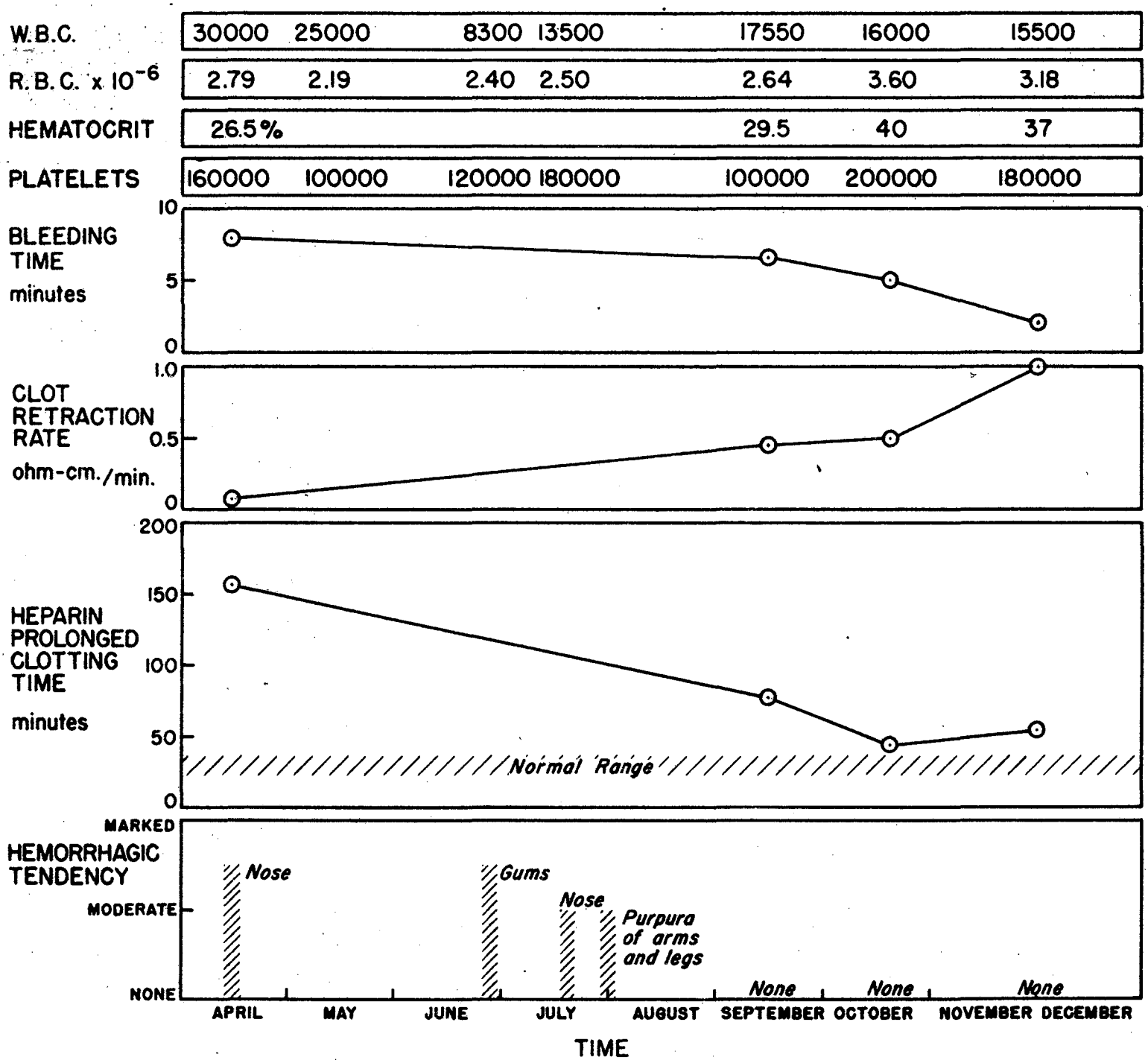


FIGURE 12

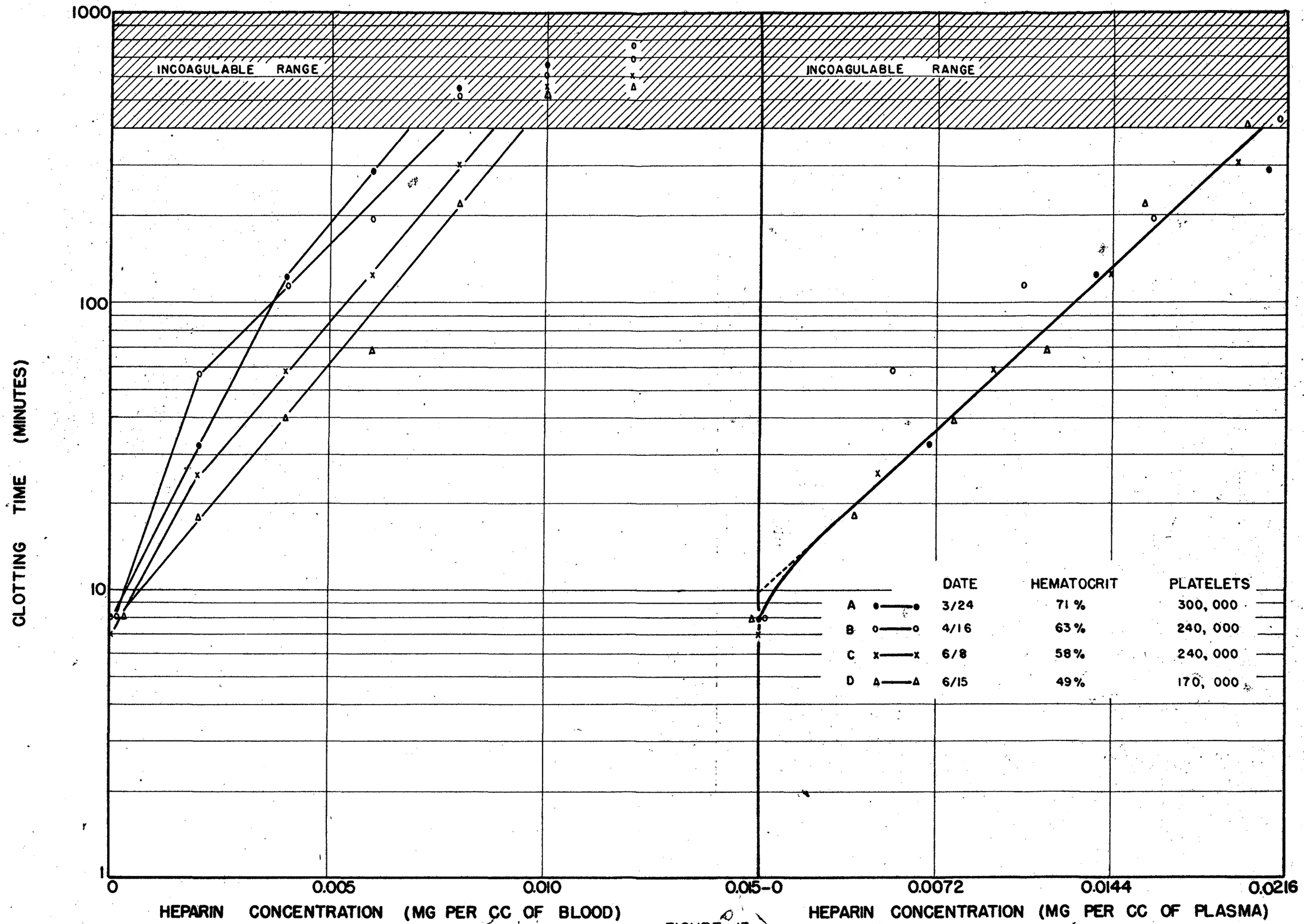


FIGURE 13

4F 2282

## The Effects of Radiation on Escherichia Coli

Robert S. Weatherwax and R. Lowry Dobson

An investigation of the effects of irradiation upon Escherichia coli, strains B and B/r, is being continued. A number of interesting observations have been made in work with ultraviolet light and with x-rays. Conventional diluting and plating techniques are being used and in experiments on the lethal effect of x-rays the definition of death has been the inability of a cell to give rise to a visible colony within 24-48 hours on nutrient agar. In experiments with ultraviolet light, it is becoming increasingly apparent that this definition is subject to some qualification.

X-rays. Unfiltered 200 Kv x-rays have been used in all of the experiments described. With cells from cultures 20-24 hours old, exponential survival curves are obtained and the x-ray dose which reduces the surviving fraction of a saline suspension of such cells to $e^{-1}$ (37 percent) is about $2 \times 10^3$ r. A few experiments indicate that the slope and possibly the shape of the survival curve may depend upon the age of the cells at the time of irradiation. As reported previously for ultraviolet light [1] and as suggested in 1941 by Rentschler, Nagy and Mouromseff [2] for ultraviolet light, it seems in these x-ray experiments that the size or the radiation sensitivity of the target area changes as the culture passes through various stages of the growth curve.

The nature of the suspending medium has a marked effect upon the resistance of the organisms. In the course of this work some experiments were performed in which the bacteria were irradiated in thin, dry films of gelatin and glycerol. The glycerol was added to facilitate the redissolving of the dried films. Dilutions of a 16-hour culture were made into a solution of 6 percent gelatin and 5 percent glycerol and measured amounts of these suspensions were dried down to thin films on the surface of cover slips. These films were irradiated, redissolved in warm saline and samples plated for counting. Under such conditions the 37 percent survival dose was some 15-20 times greater than that of the suspensions in saline alone (Figure 1).

Similar experiments with B/r are under way and it has been shown that this organism is very resistant in dried films. Doses up to $15 \times 10^3$r have failed to show any effect at all upon this organism under such conditions.

It appears that the increase in resistance could be due simply to dehydration of the cells. The removal of water might mean the removal of the mediating principle in an indirect effect or it might produce a change in the sensitivity or the size of the target area. Many virus particles in solution are known to be larger in size than the same particles in the dry state, but there does not seem to be any difference in the radiation sensitivity between the dried and the hydrated particles [3].

Changes in resistance of the magnitude reported here are not due to differences in the absorbtion of x-rays by the suspending media.

Ultraviolet Light. The recent and as yet unpublished observations by A. Kelner on the recovery of an Actinomycete from the growth inhibiting effects of ultraviolet light as a result of treatment with visible light has prompted similar investigations on E. coli. The results of this work are not entirely consistent as yet, but it can be said that there is a definite effect. A large proportion of cells, irradiated with ultraviolet light, which would normally fail to give rise to colonies prove able to

do so after treatment for a short time with an intense source of visible light. An ordinary No. 2 reflector-type photoflood lamp has proved to be a satisfactory light source when used at a distance of a few inches from the suspensions of bacterial cells. The cell suspensions are kept at room temperature by means of a water bath during the exposure (Figure 2). Further, it appears that the bacteria can be made more resistant to the inhibiting effect of ultraviolet light by giving the visible light treatment before the ultraviolet irradiation.

There are many statements in the literature to the effect that the actual death of a bacterium which has been irradiated occurs at the time of the first cell division after irradiation. There is evidence which makes it tempting to suggest that the recovery phenomenon is apparent whenever the irradiated cells are enabled to approach this division more slowly than usual. Hollaendar [4] has shown that one of the effects of the blue visible spectrum is to produce an extension of the lag phase in the bacterial growth curve. If this were the explanation of the above recovery phenomenon, it would be easier to explain why the cells are made more resistant by illumination before the ultraviolet irradiation.

Roberts in some unpublished work has shown that recovery of ultraviolet treated cells occurs if they are kept overnight at refrigerator temperatures before plating. These organisms would ordinarily have been considered dead if they had been plated and incubated immediately after irradiation, but the storage at low temperature apparently allows them to recover and to produce colonies upon subsequent plating.

Hollaendar and Duggar reported in 1938 [5] on the growth curves of E. coli after irradiation with ultraviolet light. They observed that the irradiation produced an extension of the normal lag phase of the organisms, but that this phase contained a rather sudden increase in the number of viable cells at a time when the non-irradiated control culture had not yet started to divide. This "apparent initial increase" has been cited on occasion as an example of a stimulatory effect of ultraviolet light. These growth curves were plotted from cultures growing at 32°C in dilute nutrient broth. In keeping with the trend of the above speculation, it might be suggested that this is another example of a situation in which the organisms were given an opportunity to recover to a certain extent from the inhibitory effects of the ultraviolet irradiation.

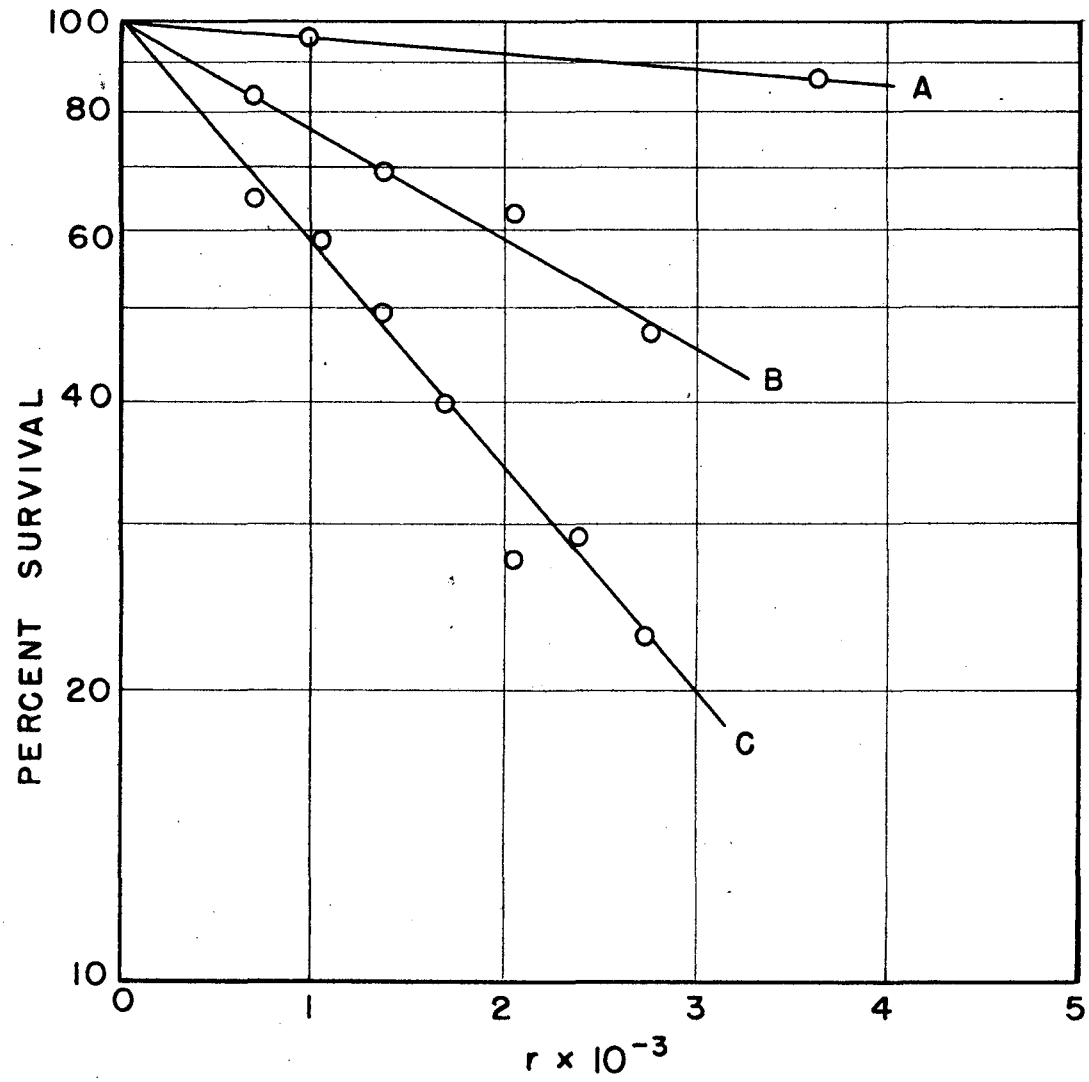


Figure 1 Survival curves of E. coli Strain B with x-irradiation. Curve C represents the survival of bacteria in saline suspension and is the average of two experiments. Curve B represents survival in 5% glycerol in saline. Curve A represents survival in a dried film of glycerol and gelatin. Additional points have been established for Curve A at greater exposures.

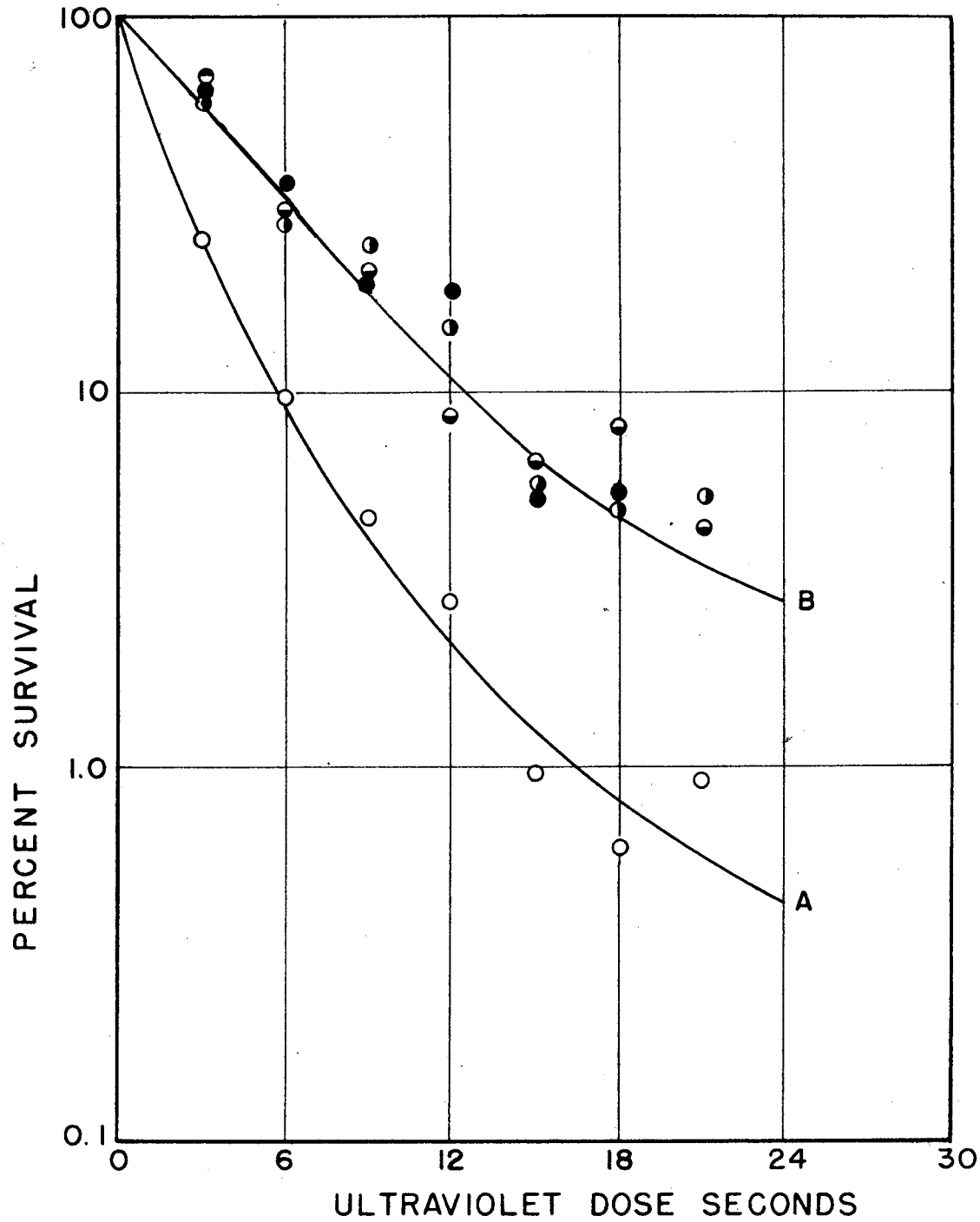


Figure 2 Recovery from effect of ultraviolet irradiation with various exposures to visible light.

Curve A represents survival curve with no illumination.
Curve B represents illumination times of 7, 14, and 21 minutes.

BIBLIOGRAPHY

1. Medical and Health Division, Quarterly Report, October, November and December, 1948, University of California Radiation Laboratory, UCRL 270.

2. Rentschler, H. C., Nagy, R., and Mouromseff, G., 1941, Bactericidal Effect of Ultraviolet Irradiation. J. Bact. 41 , 745-774.

3. Lea, D. E., and Smith, K. M., 1942, Inactiviation of Plant Viruses by γ-rays, x-rays and α-rays. Parasitology 34, 227.

4. Hollaendar, A., 1942, Albiotic and Sub-Lethal Effects of Ultraviolet Radiation on Micro-organisms. A. A. A. S., publ. No. 17, Aerobiology, 156-165.

5. Hollaendar, A., and Duggar, B. M., 1938, The Effect of Sub-Lethal Doses of Monochromatic Ultraviolet Radiation on the Growth Properties of Bacteria. J. Bact. 36, 17-37.

# III BIOLOGICAL EFFECTS OF RADIATION FROM EXTERNAL AND INTERNAL SOURCES

Robert S. Stone - in Charge

Project 48C

University of California Medical School

## Hematological Effects of Total Body Irradiation

B. V. A. Low-Beer

This report is concerned with hematological effects of radioiodine ($I^{131}$) on patients. Two groups of patients have been observed. In one group, including eight individuals, seven had Graves' disease and one had chronic thyroiditis. A second group of eight individuals had carcinoma of the thyroid. Observations were made over periods from three months to one and a half years. Carrier-free $I^{131}$ was administered orally. The doses varied from "test" doses to therapeutic doses. All total doses were given in multiple fractions of different size at different intervals. In the tables the individual doses are shown and the time of administration is given. Clinical management and radioiodine treatment of these patients including uptake and excretion studies are being carried on by Dr. Earl R. Miller.

It was intended that blood counts would be made just before treatment and once a week for eight weeks, once a month for three months and once every three months thereafter, if the doses exceed 10 millicuries. For patients who received less than 10 millicuries in a single dose, blood counts would be taken once every two weeks for eight weeks and then every two months thereafter. It was not possible to carry out this schedule exactly in all cases.

In previous reports criteria for evaluating hematological changes have been established. In accordance with these, the following fluctuations are considered significant. Rbc, 15 percent ±; Hb, 15 percent ±; platelets 50 percent ±; WBC, 25 percent ±; monocytes 100 percent ±.

No attempt has been made here to evaluate changes in lobation index because the significance and validity of reported findings is still undetermined. We have also confined our observations to quantitative changes and have not attempted to interpret changes in staining characteristics of the cells which were observed occasionally.

In the eight cases shown in Table I, total doses of $I^{131}$ varied from 1006 microcuries administered over a three month period, to 11,600 microcuries administered over an eight month period. It is striking that in the cases with Graves' disease the only significant change within the first three months of the initial

dose, occurred in the white blood cell count of one patient. This is the patient who received the lowest total dose of $I^{131}$ of the group. The other patient whose blood count showed significant changes within the first three months was the patient with chronic thyroiditis who had a thyroidectomy a few hours after administration of $I^{131}$, only one patient with Graves' disease showed any significant change in blood count. The patient with chronic thyroiditis also showed significant blood count changes during this period. Only two patients of this group were observed longer than nine months. Neither showed any significant change in blood count after nine months.

In the eight cases in Table II, total doses of $I^{131}$ varied from 1600 microcuries given in one dose, to 130,000 microcuries given over a period of 14 months. One single dose however, was 100,000 microcuries. Of the four patients who received total doses in excess of 50,000 microcuries, the only one who showed significant changes in blood count during the first three month period after initiation of treatment was the patient who received the lowest total dose. One patient, J.L., received a single dose of 100,000 microcuries in addition to 30,270 microcuries that he had received in divided doses during the preceding six months. He showed no change in his blood prior to this single large dose, but one month after receiving it there was a significant increase in his white blood cell count. In all, five patients showed significant changes in blood count during the second period of five months. Only three patients were observed longer than eight months. All of these patients had received total doses in excess of 50,000 microcuries and all three showed some significant change in their blood cell counts after eight months of initiation of treatment. There is no correlation between the occurrence of significant changes in the blood and any one of the following factors: (a) the size of the total dose (b) the size of the individual dose (c) the amount of radioiodine concentrated in the thyroid (d) the rate of loss of radioiodine from the body (urinary excretion).

The foregoing is a preliminary report. We are at present computing the total body radiation dose due to beta as well as gamma rays from $I^{131}$. This calculation is of particular interest in those cases where very large doses of $I^{131}$ are given and where selective uptake by the thyroid is low.

TABLE I

PATIENTS WITH GRAVES' DISEASE TREATED WITH $I^{131}$

| Patient | Uptake Average | Doses $I^{131}$ | OBSERVATION TIME | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 - 3 months | | | | 4 - 8 months | | | | More than 8 months | | | |
| | | | RBC | HB | PLATE | WBC | RBC | HB | PLATE | WBC | RBC | HB | PLATE | WBC |
| E. R.<br>F 28 yrs | 70% | 3950 μc 11-12-47<br>3150 μc 12-15-47<br>261 μc 3-24-48<br>3000 μc 3-25-48<br>232 μc 6-2-48<br>1000 μc 6-4-48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| R. P.<br>f 25 yrs | 70% | 246 μc 4-12-48<br>2977 μc 4-14-48<br>135 μc 6-9-48<br>260 μc 1-11-49 | 0 | 0 | 0 | 0 | 0 | dec. | 0 | 0 | 0<br>Blood picture shows evidence of mild secondary anemia. | 0 | 0 | 0 |
| V. M.<br>f 15 yrs | 75% | 238 μc 4-12-48<br>2700 μc 4-14-48<br>239 μc 6-15-48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | |
| E. H.<br>m 41 yrs | 60% | 250 μc 11-3-48<br>4000 μc 11-12-48<br>167 μc 12-27-48 | 0 | 0 | 0 | 0 | | | | | | | | |
| R. R.<br>f 15 yrs | 75% | 150 μc 12-20-48<br>4246 μc 12-23-48<br>200 μc 2-14-49 | 0 | 0 | 0 | 0 | | | | | | | | |
| F. W.<br>f 34 yrs | 45% | 190 μc 1-10-49<br>410 μc 1-17-49<br>156 μc 3-28-49<br>250 μc 4-5-49 | 0 | 0 | 0 | inc.<br>dec. | | | | | | | | |
| E.C.<br>f 39 yrs | 60% | 271 μc 1-25-49<br>1690 μc 1-28-49<br>162 μc 3-21-49 | 0 | 0 | 0 | 0 | | | | | | | | |
| N. S.<br>f 54 yrs<br>chronic thyroiditis | 32% | 2072 μc 11-2-48<br>Total thyroidectomy 11-2-48 | 0 | 0 | inc. | inc. | 0 | 0 | dec. | inc. | | | | |

TABLE II

PATIENTS WITH THYROID CARCINOMA TREATED WITH $I^{131}$

| Patient | Uptake Average | Doses $I^{131}$ | OBSERVATION TIME 0 - 3 months | | | | 4 - 8 months | | | | More than 8 months | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | RBC | HB | PLATE | WBC | RBC | HB | PLATE | WBC | RBC | HB | PLATE | WBC |
| R.E.<br>m 24 yrs | Max. 20% | 2000 µc 1-7-47<br>7000 µc 1-22-47<br>50,000 µc 6-9-47 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | inc<br>dec |
| J.L<br>m 18 yrs | Max. 26%<br>Marked myx-edema after 100,000 µc | 2000 µc 10-16-47<br>670 µc 11-20-47<br>25,000 µc 12-9-47<br>480 µc 1-21-48<br>150 µc 2-16-48<br>2000 µc 3-29-48<br>100,000 µc 4-16-48<br>235 µc 7-8-48<br>1067 µc 8-4-48<br>970 µc 12-14-48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | inc. 1 mo. after 100,000 µc | 0 | 0 | dec. | dec.<br>PMN down, L up |
| R.H.<br>f 59 yrs | 5% | 2000 µc 1-12-48<br>2168 µc 2-16-48<br>2000 µc 3-20-48<br>89,000 µc 4-19-48<br>250 µc 7-14-48<br>1000 µc 9-8-48<br>1000 µc 11-16-48 | 0 | dec. | 0 | dec. | 0 | 0 | 0 | dec. | 0 | 0 | 0 | dec. Drop in WBC<br>Progressive |
| L.C.<br>f 29 yrs | 6%<br>Marked myx-edema after 50,000 µc | 2000 µc 4-26-48<br>53,900 µc 5-14-48<br>250 µc 7-14-48<br>990 µc 8-11-48<br>1000 µc 9-7-48<br>998 µc 3-2-49<br>1063 µc 3-11-49<br>1049 µc 3-18-49 | 0 | 0<br>General condition deteriorating | 0 | 0 | dec. | dec. | dec. | 0 | | | | |
| M.C<br>f 11 yrs | 18% | 1600 µc 11-2-48 | 0 | dec. | 0 | inc. | 0 | dec. | 0 | dec. | | | | |
| J.W.S.<br>m 66 yrs | -5% | 2000 µc 9-9-48<br>30,000 µc 9-10-48<br>38,200 µc 9-30-48 | 0 | 0 | 0 | 0 | | | | | | | | |
| M.G.<br>m 20 yrs | 21% | 1575 µc 11-2-48<br>257 µc 9-13-48 | 0 | 0 | 0 | 0 | 0 | dec. | 0 | 0 | | | | |
| E.H.<br>f 54 yrs | Neglibible | 2194 µc 8-4-48<br>568 µc 9-13-48 | 0 | 0 | 0 | 0 | | | | | | | | |

## Metabolism & Effects of Radio-Iodine ($I^{131}$)

Earl R. Miller

Studies of the uptake of radioiodine in various disease of the thyroid has continued. Where indicated, radioiodine has been used in the treatment of patients with Graves' disease and those with cancer of the thyroid.

Since the start of this project 287 patients have been given radioiodine, either for tracer or therapeutic purposes or both and of this number 43 have been seen in the past three months. However, no new facts have been elucidated in this period and a future report will therefore be held up until the next quarterly report, when it is hoped that sufficient important data will be accumulated to justify a report.

# IV HEALTH CHEMISTRY AND PHYSICS

## Health Chemistry

N. B. Garden

Monitoring. Continuing application of the philosphy of "No Contamination--No Exposure" is reducing the routine monitoring to a minimum. Monitors's reports have been simplified by the use of standard forms. In consequence, the monitor is able to spend more of his time studying the individual problems of the chemists. The monitor is learning to prescribe standard Health Chemistry equipment to reduce further the radiation hazards to personnel and to lower research contamination levels.

The "wrap-on" finger films are continuing to find favor and their use is increasing. An effort is being made to find a commercial supply since the rings now in use must be made up by the monitor from regular-width dental film. It has been necessary to increase the number of instruments available to the monitor because the number of workers with radioactivity has increased.

Research and Development. As the monitors call to the attention of the chemists the equipment, standard and special, that can be supplied through Health Chemistry Division, there has been a greater and greater demand and the Research and Development Section has been taxed to capacity.

Much progress has been made on preparations for the special Hanford sample, scheduled for May, 1949. The required shielding for "shine" in the cave has been completed. The remote chemistry unit is well under way and other phases are rapidly shaping up. The slug opener has been completed.

Special projects completed in this quarter include: a special three-months' bombardment for S. G. Thompson on the 60 inch cyclotron, three portable lead caves with lead glass brick windows to provide one-inch lead shielding for the standard gloved boxes, liquid target holder shielding and transport from the 60 inch cyclotron, microinterceptor for active bombardments on the 60 inch cyclotron, hood for vacuum system containing plutonium, four 18- x 36 inch lead glass panels of one-quarter inch thickness for beta shielding on the standard gloved boxes, a "safe bottle" for storage and transfer of plutonium solutions, and three gloved boxes for special uses. In addition thirteen standard boxes were completed in this quarter.

Transportation, Disposal, Storage and Salvage. Plans are nearing completion for the installation of a pneumatic tube transport system for short-lived targets from the 184 inch cyclotron to a chemistry laboratory in Building 5. This system will place a target in the hands of the chemist in less than a quarter of a minute after the bombardment is completed. In addition, exposure to the personnel handling the targets will be reduced to practically zero. Salvage of a considerable number of gloved boxes and auxiliary equipment is still awaiting completion of a building to house the decontamination chamber.

## Health Physics

B. J. Moyer

Radiation Field of 184 inch Cyclotron with 350 Mev Proton Operation. Surveys have been performed with instruments of the type mentioned in the preceding quarterly report. Results are dependent upon beam direction and the nature of the target to a much greater extent than is the case with deuteron operation.

The surveys immediately indicated the shielding "weakness" of the 5 ft. concrete doors. This has been in large measure corrected by building into each door aperture a concrete maze.

With present maximum beam (1/2 to 1 micro-ampere) in the normal direction on a one-half-inch-thick Be target, the surveys show results as follows:

Fast neutrons, 0.5 - 15 Mev.

Energy flux values at 5 ft. from floor range from 6 to 180 Mev/cm$^2$ sec. The high values are related to azimuths where primary neutrons spray the inside of the shielding in large number.

At 33 ft. from the floor the values are about twice as high.

Slow neutrons.

The flux density ranged from 20 to 60 neutrons/cm$^2$ sec, and did not display any pronounced variations.

Ionization in plastic-wall chamber.

Expressed in milli-reps per hour, the values at 5 feet from the floor fell between 0.2 and 6. The latter figure pertains to a region outside the north shielding door where the level was subsequently reduced to about 3 by the maze built into the doorway.

At 30 feet the intensity is increased by a factor of two to three.

When the cyclotron beam is reversed, so that the primary neutron spray from the target is directed northward (instead of into the concrete "igloo" on the south side) the intensity of fast neutrons and m rep/hr exceed tolerance values within a certain azimuth in the building, and it is necessary to prevent prolonged work in this region.

Some data have been gathered on the penetration of concrete by the high-energy neutrons from proton operation. For ordinary structural concrete the broad-beam attenuation of the neutrons at 0$^{\circ}$ shows a 17" to 18" half-value thickness. This has been measured with three types of detection, namely, a bismuth fission chamber, carbon detectors, and an ionization chamber.

Indium foils placed at intervals through the 10 ft. concrete walls out of the azimuth of the forward cone show an attenuation with half-value of thickness of 11" to 13", except for transition effects in which equilibrium radiation is attained.

These observations suggest that a measurable flux of primary, high-energy neutrons may penetrate the 10 foot walls. A bismuth fission counter in continuous operation is collecting data on flux of neutrons with energy exceeding 50 Mev. Present data indicate that the value may be of the order of one per $cm^2$ per sec.

Notes on Radiation Field of Linear Accelerator. Twenty-two location films are regularly posted in representative locations throughout the building. In a typical week of operation only seven of these receive x-ray dosages under 0.7 roentgens. The dosages on other films extend as high as 5.0 roentgens.

In the vicinity of the target, just outside the barriers, under conditions of large proton beams, the fast neutron energy flux is approximately at the tolerance level (200 $Mev/cm^2sec$). But because of congested conditions it is very difficult for experimenters to avoid occasionally entering regions of higher flux.

The linear accelerator is presently shut down to allow overhaul including measures to reduce x-ray intensity, and the installation of adequate shielding and a target area enclosure.

Statistical Summary of Monitoring Program.

A. Survey instruments maintained

| | | |
|---|---|---|
| 1. | B-V ionization chambers | 28 |
| 2. | Victoreen 263 meters | 19 |
| 3. | I.D.L. protable survey meters | 12 |
| 4. | Cutie Pies | 2 |
| 5. | Recording V-intensity meters | 10 |
| 6. | Victoreen Proteximeters | 3 |
| 7. | Fast neutron proportional counter | 1 |
| 8. | Balanced chamber slow neutron survey instrument | 1 |
| 9. | Special tissue wall survey instrument | 1 |

B. Personnel meters in use

| | | |
|---|---|---|
| 1. | Number of people with film badges | 1200 |
| 2. | Total man days coverage with pocket chambers | 2922 |
| 3. | Total man days coverage with pocket dosimeters | 4840 |
| 4. | Total man days coverage with pocket chambers, s.m. | 3163 |

C. Cases of weekly exposure above .3 r -119

| | | |
|---|---|---|
| 1. | Linear accelerator | 85 |
| 2. | 184 inch area | 3 |
| 3. | 60 inch area | 16 |
| 4. | Chemistry | 15 |

Cases of weekly exposure above .5 r -39

| | | |
|---|---|---|
| 1. | Linear accelerator | 28 |
| 2. | 184 inch area | 1 |
| 3. | 60 inch area | 7 |
| 4. | Chemistry | 10 |

Cases of weekly exposure above .7 r -18

| | | |
|---|---|---|
| 1. | Linear accelerator | 14 |
| 2. | 184 inch area | 0 |
| 3. | 60 inch area | 1 |
| 4. | Chemistry | 3 |

Cases of weekly exposure above 1.0 r -6

| | | |
|---|---|---|
| 1. | Linear accelerator | 5 |
| 2. | 184 inch area | 0 |
| 3. | 60 inch area | 0 |
| 4. | Chemistry | 1 |

Cases of weekly exposure above 1.5 r but not over 1.7 r -1

| | | |
|---|---|---|
| 1. | Linear accelerator | 1 |

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