to be able to depend on the presence of lipemia the food was not given in the afternoon as usual but reserved until morning, or sometimes an extra meal consisting of 100 gms each of salmon bread, Klim and cod liver oil was fed from two to four hours before sampling.

Both blood samples were taken from the same jugular vein, the needle being left in place. The injected material was also introduced through this needle. The degree of lipemia was not quantitated except roughly as follows: 0 – water clear plasma; 1 + – slight turbidity; 2 + definite lipemia; 3 + sufficient lipemia to obscure the meniscus of the plasma completely; 4 + – same as 3 + but with frank lipid layer on top the plasma layer.

In Table 1 is shown a series of reactions in which heparinized whole blood from a number of donor dogs was injected, as well as plasma from heparinized blood. Two makes of heparin were used, those experiments marked with * were carried out with a preparation obtained from the Hynson and Westcott Company of Baltimore, while those marked ** were done with material of considerably greater specific potency (1 mgm = 110 units) obtained from the Connaught Laboratories of Toronto. In all experiments in which heparinized whole blood or plasma was administered the lipemia was abolished. The reaction did not occur on the injection of washed red cells derived from heparinized blood nor after injection of any fractions derived from citrated blood.

It was finally found that the same amount of heparin (**2.4 mgm) as used in the earlier experiments when dissolved in saline and given by vein would in itself abolish the lipemia.

Addition of 5 mgm of heparin to 5 ml of lipemic plasma *in vitro* with mixing showed no reaction on standing. Neither did mixing of heparin with non-lipemic plasma and subsequent mixing with lipemic plasma result in the clearing of the latter.

The time interval of 5 minutes elapsing between the injection of the heparinized material and sampling is more than necessary for the reaction to occur. In three of the experiments shown in Table 1, samples were taken at ½, 1, 1.5, 2 and 3 minutes as well as 5 minutes. In each of these instances the lipemia was practically absent in the recipient's blood at the end of 1 minute.

TABLE 1

LIPEMIA BEFORE AND AFTER ADMINISTRATION OF HEPARIN

| Date | Dog | | Material introduced | Degree of lipemia | | Donor source |
|---|---|---|---|---|---|---|
| | | | | Initial | Final | |
| 1/8 | 40–115 | * | 20 ml heparinized whole blood | 3 + | 0 | 39–266 |
| 1/20 | 41–164 | ** | " " " " | 3 + | 0 | 1–J |
| 1/19 | 1–J | ** | " " " " | 3 + | 0 | 39–266 |
| 1/4 | 1–K | * | " " " " | 3 + | 0 | 39–266 |
| 1/8 | 1–K | * | " " " " | 4 + | 0 | 39–266 |
| 1/19 | 40–115 | ** | 20 ml heparinized plasma | 2 + | 0 | 1–J |
| 1/19 | 41–164 | ** | " " " | 4 + | 0–1 + | 39–266 |
| 1/22 | 39–266 | ** | " " " | 4 + | 1 + | 38–137 |
| 1/20 | 39–196 | ** | " " " | 4 + | 0–1 + | 40–149 |
| 1/13 | 1–J | | 20 ml citrated whole blood | 2 + | 2 + | pooled |
| 1/13 | 38–137 | | " " " | 3 + | 3 + | " |
| 1/18 | 39–196 | | " " " " | 3 + | 3 + | 39–266 |
| 1/13 | 40–115 | | 25 ml citrated plasma | 4 + | 4 + | pooled |
| 1/14 | 39–57 | | " " " | 4 + | 4 + | " |
| 1/22 | 39–266 | * | 20 ml washed heparinized cells | 4 + | 4 + | 38–137 |
| 1/19 | 40–149 | * | " " " " | 3 + | 2 + | 39–266 |
| 1/13 | 1–K | | 25 ml citrated washed cells | 2 + | 2 + | pooled |
| 1/18 | 1–K | | " " " " | 4 + | 4 + | " |
| 1/23 | 40–115 | ** | 20 ml citrated plasma (itself lipemic) to which was added 250 units of heparin | 2 + | 0 | 39–266 |
| 1/23 | 41–164 | ** | 250 units of heparin in 5 ml saline | 2 + | 0 | .... |
| 2/11 | 39–57 | ** | " " " " " " " " | 4 + | 0 | .... |
| 1/28 | 40–115 | ** | " " " " " " " " | 1 + | 0 | .... |
| 2/1 | 38–137 | ** | " " " " " " " " | 3 + | 0–1 + | .... |
| 2/11 | 39–266 | ** | " " " " " " " " | 3–4 + | 0 | .... |

On a basis of the data presented here it would not be wise to speculate as to the nature of the mechanism involved in the abolishment of lipemia from the blood of dogs as a result of the injection of moderate amounts of heparin. Fractional lipid analyses of plasma taken before and after injection of heparin are being carried out and will be reported at a later date.

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## PRODUCTION AND TREATMENT OF GRANULOCYTOPENIA AND ANEMIA IN RATS FED SULFONAMIDES IN PURIFIED DIETS

THE production of granulocytopenia and anemia in rats through the use of sulfanilylguanidine or succinyl sulfathiazole in purified diets and treatment of the animals with whole dried liver or liver fractions have been reported from this laboratory.[1] The pres-

[1] S. S. Spicer, F. S. Daft, W. H. Sebrell and L. L. Ashburn, *Pub. Health Rep.*, 57: 1559, 1942.

ent study concerns the production and treatment of similar blood dyscrasias in rats receiving sulfathiazole, sulfadiazine or sulfanilamide.

## PRODUCTION

Albino rats were weaned at 21 to 27 days and placed on one of two experimental diets. One hundred and one rats were given a diet composed of glucose ("Cerelose") 72 per cent., "vitamin-free" Smaco casein 18 per cent., cod liver oil 2 per cent., cottonseed (Wesson) oil 3 per cent., salt mixture No. 550[1] 4 per cent. and either sulfathiazole, sulfadiazine or sulfanilamide at a level of 1 per cent. Twenty-six additional rats received one of these three sulfonamide drugs in a similar diet, except that it contained 25 per cent. Smaco casein and 65 per cent. glucose ("Cerelose"). Each rat received a daily supplement of 100 micrograms of thiamine hydrochloride, 200 micrograms of riboflavin, 100 micrograms of pyridoxine hydrochloride, 200 micrograms of calcium pantothenate, 1 mg of niacin and 10 mg of choline chloride. The rats were weighed three times weekly and after weight gain had ceased or when the animals appeared sick, blood counts were made and in some of the animals were repeated at irregular intervals. The techniques were the same as those previously employed.[1] The term "blood dyscrasia" is used in this report to denote a "severe granulocytopenia" or a "severe anemia" or both combined. "Severe granulocytopenia" is used to indicate a total polymorphonuclear granulocyte count of less than 150 cells per cu mm. The term "severe anemia" is used to indicate hemoglobin or hematocrit values of less than 7.5 grams per 100 cc or 25 cc per 100 cc, respectively. Milder degrees of granulocytopenia and anemia are not considered in this report.

From the data obtained, no marked differences have been noted between the incidence of blood dyscrasias in the rats given 18 per cent. Smaco casein and those given 25 per cent. Smaco casein in these diets; also the response to treatment has been similar. The data concerning both groups therefore have been considered together. Of the 127 animals on experiment, 22 were excluded from consideration because they died without blood counts having been made. Fifty-two animals developed a blood dyscrasia. The remaining 53 animals not showing such severe blood changes have been under observation from 39 to 169 days. Twenty-one of these animals have died and the remaining 32 have been sacrificed. Blood dyscrasias have been found as early as the tenth experimental day and as late as the eighty-sixth. The data in Table I do not include less severe degrees of granulocytopenia and anemia which were observed frequently.

It is to be noted that under these experimental conditions, the incidence of blood dyscrasias was very much less in the sulfanilamide group than in either the sulfathiazole or sulfadiazine groups. For these groups of animals the incidence of severe granulocytopenia was greater in those receiving sulfathiazole than in those receiving sulfadiazine, while the reverse

TABLE I

THE OCCURRENCE OF SEVERE GRANULOCYTOPENIA AND ANEMIA IN RATS INGESTING A SULFONAMIDE-CONTAINING, PURIFIED DIET

| Sulfonamide contained in the diet | Total number of rats studied | Number of rats with a blood dyscrasia (severe granulocytopenia or severe anemia or both) | Number of rats with a total polymorphonuclear granulocyte count of 0–150 cells/cu mm | Number of rats with a hemoglobin or hematocrit of less than 7.5 gms/100 cc or 25 cc/100 cc respectively |
|---|---|---|---|---|
| Sulfathiazole ..... | 34 | 28 | 22 | 10 |
| Sulfadiazine ..... | 36 | 22 | 9 | 16 |
| Sulfanilamide ..... | 35 | 2 | 2 | 1 |

was true of the incidence of severe anemia. Blood smears from severely anemic animals usually revealed marked abnormalities in the size, shape and staining reactions of the red blood cells and an increased number of nucleated forms. In the animals with severe granulocytopenia, a leucopenia with a mean value of about 4,000 cells per cu mm was usually present.

## TREATMENT

Of the 52 animals that developed a blood dyscrasia, 30 were not treated and died. Of the remaining 22 animals, 17 were treated for a severe granulocytopenia, four for a severe anemia and one for both conditions combined. Fifteen of the 18 animals with granulocytopenia[2] were treated by the daily oral administration of 1 cc of an aqueous suspension containing 0.25 gm per cc of Wilson's solubilized liver,[3] or smaller amounts of more refined liver fractions; three were given a daily oral supplement of 1 gm of whole dried brewers' yeast. The treatment period was four days, during which time the animals continued to eat the sulfonamide-containing diet. Counts were made immediately before treatment and again at the end of four days of treatment. Five of the liver-treated animals failed to survive for more than three days. Recounts of the 13 animals which did survive the treatment period revealed a significant response in every instance. Table II contains the values for the liver-treated group before and after treatment. Five rats were severely anemic and four of them[4] were

[2] The animal with granulocytopenia and anemia combined is included here.

[3] Furnished through the courtesy of Dr. David Klein, Wilson Laboratories.

[4] The animal with granulocytopenia and anemia combined is included here.

treated with 1 cc of Wilson's solubilized liver suspension for each of 10 successive days. The data for these animals are given in Table II, and as noted all showed some response at the end of five days; near normal levels were observed after 10 days of treatment. The one anemic animal not included in the table had an initial hemoglobin value of 6.5 gms per 100 cc. After 10 days of treatment with another fraction, it had fallen to a level of 2.3 grams per 100 cc, the hematocrit remaining unaltered at 18 cc per 100 cc. Treatment with the suspension of Wilson's solubilized liver was then given for 12 days. The hemoglobin level rose to 11.5 grams per 100 cc and the hematocrit to 56 cc per 100 cc. All the 14 animals treated with liver fractions and the three treated with brewers' yeast showed a resumption of growth and a marked clinical improvement.

TABLE II

BLOOD VALUES SHOWING A RESPONSE OF SEVERELY GRANULOCYTOPENIC OR ANEMIC RATS TO TREATMENT WITH LIVER FRACTIONS

| | Number of experimental rats | Before treatment | | Number of days of treatment | After treatment | | Normal values cited in literature | |
|---|---|---|---|---|---|---|---|---|
| | | mean | range | | mean | range | mean | range |
| Total polymorphonuclear granulocytes per cu mm | 10 | 29 | 0–103 | 4 | 3,203 | 1,550–5,760 | 3,465 | 1,200–6,825 |
| Percentage of polymorphonuclear granulocytes | 10 | 0.8 | 0–3 | 4 | 30.1 | 21–41 | 29.9* | 15–45.5* |
| Total white blood cells per cu mm | 10 | 3,808 | 550–7,320 | 4 | 10,550 | 4,950–16,950 | 11,590* | 8,000–15,000* |
| Hemoglobin in gms per 100 cc | 4‡ | 7.0 | 6.4–7.4 | 5 | 9.1 | 8.2–9.7 | 15.6* | 14.0–17.2* |
| | | | | 10 | 13.6 | 13.0–14.1 | | |
| Red blood cell volume (Hematocrit) in cc per 100 cc | 4‡ | 24 | 21–27 | 5 | 35 | 33–39 | 50† | ..... |
| | | | | 10 | 45 | 41–47 | | |

* R. A. Scarborough, *Yale Jour. Biol. and Med.*, 3: 267, 1931.
† A. J. Creskoff, T. Fitz-Hugh, Jr., and E. J. Farris, in "The Rat in Laboratory Investigation," edited by J. G. Griffith and E. J. Farris, p. 351. Philadelphia: J. B. Lippincott Co., 1942.
‡ One additional animal is discussed in the text.

The mechanism of action of the sulfonamide drugs in producing these blood dyscrasias as well as other toxic manifestations is obscure. It has been suggested[1] that there may be an indirect toxicity such as an interference with tissue enzyme systems. Also, the possibility was considered that such an indirect toxicity, a direct toxicity and a reduction of intestinal synthesis of essential factors might all be involved.

## SUMMARY

(1) A severe granulocytopenia or anemia, or both, have been produced in rats fed sulfathiazole, sulfadiazine or sulfanilamide at a 1 per cent. level in purified diets.

(2) Treatment with certain liver fractions orally has succeeded in correcting the granulocytopenia in four days and the anemia in about 10 days in spite of continued ingestion by these animals of the sulfonamide-containing diet.

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# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## THE DETERMINATION OF CELL VOLUME AND HEMOGLOBIN ON THE SAME DROP OF BLOOD

AN adequate clinical description of the erythrocytes in blood requires the determination of the cell volume, the hemoglobin content and the cell count.

A method for determination of cell volume, called the cell opacity method, has been described recently.[1] It involves the measurement in a photoelectric colorimeter of the transmission of light at a wave length of about 660 mμ through a suspension of blood in citrate solution. The cell opacity thus recorded is proportional to the cell volume, which can be read from a line or table showing this relationship. This method requires only 5–25 cmm of blood.

A method for the determination of hemoglobin by the measurement in a photoelectric colorimeter of the transmission of light of about 540 mμ has been described by Evelyn.[2] This determination requires a similar amount of blood.[3]

[1] A. T. Shohl, *Jour. Lab. and Clin. Med.*, 25: 1325, 1940.

[2] K. A. Evelyn, *Jour. Biol. Chem.*, 115: 63, 1936; also K. A. Evelyn and H. T. Malloy, *ibid.*, 126: 655, 1938.