with the inhibition of growth was intensified hyalinization of the matrix, but no disturbances in calcification were noted. In the metaphysis, there was deficient formation of osteoblasts leading to a decrease in the number and size of primary spicules. Vascularization and ossification of the shaft were likewise decreased. The decreased growth processes in the epiphyseal cartilage are comparable to those observed in growing rats kept on pantothenic acid-deficient diet;[2] they are compatible with and account for the reduced growth rate of mice thus treated.[1] The skeletal changes observed in this series of mice are different from those seen in animals kept on a quantitatively reduced but qualitatively adequate diet. In the latter, growth processes proceed at a decreased rate;[4] ossification may be increased in spite of the decreased growth rate, and the bone marrow undergoes serous atrophy. In pantothenic acid deficiency, ossification ceased with the proliferation and development of the cartilage, and no changes in the bone marrow occurred.

[4] Silberberg, M., and Silberberg, R., *Arch. Path.*, 1940, **30**, 675.

In the male, these changes were accentuated after only one week, whereas in the female the growth inhibition reached a similar degree after 2 weeks. After 4 weeks, animals of both sexes showed about the same changes in the growth zones. Since, at this age, the normal growth rate in males is greater than that of the females, it is not surprising that the male responded more readily to the vitamin deficiency than the female. The skeletal tissues of the adult mouse are not as dependent on this vitamin as those of the growing animal.

Summary. In growing mice, pantothenic acid is essential for the growth of the skeleton. Deficiency of this vitamin causes inhibition of growth and endochondral ossification. The male reacts more quickly to lack of pantothenic acid than the female. On feeding an adequate stock diet subsequent to a prolonged deficiency, growth of cartilage and ossification are resumed rapidly and may surpass in intensity the processes seen in normal nondeficient animals. In the adult mouse, skeletal changes were not observed under the influence of pantothenic acid deficiency.

15609

Tests for Hepatic Dysfunction of Mice.*

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Attempts to transmit infectious hepatitis and homologous serum jaundice to laboratory animals have been made by several investigators with results that are negative or inconclusive. In this laboratory we have tried to infect mice with human material derived either from spontaneous cases of infectious hepatitis or from artificially-induced serum jaundice. These experiments are still in progress.

* We thank Dr. H. A. Schneider for his help in the studies here reported on *S. enteritidis* and dietary influences. We are also indebted to Dr. R. E. Shank for his cooperation.

In the course of the latter work it was thought that mice may develop experimentally an inapparent disease with hepatic damage, without showing objective signs. In such a case it would be advantageous (a) to recognize the condition and (b) to follow the course of the damaged liver without sacrificing the animal to observe the lesions. Also, if blind passages were attempted, the knowledge of the state of the hepatic function might give an indication as to the best time to carry out such passages. For these reasons we undertook an investigation of tests of hepatic dysfunction in Swiss mice,

applying some of the methods used in clinical medicine.

Owing to the large numbers of animals used, as well as to the technical difficulties, such as obtaining blood serum, these tests had to be modified. Some of the methods, such as those involving collection of urine or feeding of known quantities of materials, were eliminated as impractical. A selection was made of the remaining tests, chiefly on the basis of their practicability in the mouse. The following were therefore investigated: retention of bromsulphalein; bilirubin in the blood; thymol-turbidity; plasma-coagulation time, and vaginal-smear cytology. Tests based on vaginal-smear cytology, although not used in human beings, were studied because an impairment in the ability of a damaged liver to inactivate estrogens[1] and the occurrence of an increased amount of the latter in the circulation of males with hepatic diseases[2] were recently reported.

For each of these tests the normal value or base line was established in control mice. Hepatic lesions were induced in mice by several means, such as by chemical injury with carbon tetrachloride, phosphorus and allyl formate; by bacterial infection with *Salmonella enteritidis;* by injection of hepatic tissue autolysates, and by starvation. The possible lesions that may be induced through dietary factors are now being investigated. Of all the tests employed retention of bromsulphalein proved to be the most dependable and is therefore reported in detail. The types of hepatic lesions in mice are presented first, followed by the description of the tests and the results obtained with them.

Hepatic Lesions in Mice. Carbon Tetrachloride. The toxic effect of carbon tetrachloride on the liver is well known. We followed the method of Cameron and Karunaratne,[3] who studied its toxicity in rats, although the mice were given larger amounts of the chemical per unit of body weight. Mice were injected subcutaneously with a mixture of 9 parts of carbon tetrachloride and 1 part of mineral oil. The amounts varied from 0.2 to 1 cc which represented from 1 to 5 cc of carbon tetrachloride per 100 g of body weight. Immediately after injection, mice became ill with cyanosis and dyspnea, a condition lasting from 10 to 30 minutes. With the larger doses of the chemical (0.5 to 1 cc), all mice appeared sick 24 hours later and about 25% of them died; within the next 4 or 5 days half the number succumbed. The survivors gradually improved and within one week after injection were completely recovered. With the smaller doses (0.2 or 0.3 cc), the symptoms were less pronounced and about 20% died.

Histological examinations of the liver 1, 2, 4, 6, 10, 15 and 27 days after the injection of carbon tetrachloride revealed lesions comparable to those described by Cameron and Karunaratne[3] in rats. At 24 hours there was a considerable degree of necrosis located about the central vein, involving at times most of the hepatic section. Periportal hepatic cells were not visibly damaged. This picture remained for another day or 2 after which new growth of hepatic cells occurred, as indicated by the presence of mitotic figures, along with progressive diminution of necrotic areas. At about the 6th day there was a great deal of diffuse cellular infiltration (polynuclear and mononuclear cells), necrotic areas were greatly reduced in size, and restoration of the architecture of the hepatic-cell cords was in progress. By the 10th day the appearance was practically normal.

Phosphorus. The administration of phosphorus to mice in order to induce hepatic lesions was not found practical. Phosphorus was given either by mouth or by subcutaneous injection, as a commercial rat-poison preparation containing yellow phosphorus. Neither route was successful. Mice refused as a rule to eat phosphorus-contaminated food, and when injected subcutaneously, phosphorus caused the development of local lesions with suppuration and the substance probably failed to reach the liver. As a consequence, only a limited number of mice were affected.

1 György, P., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 344.

2 Gilder, H., and Hoagland, C. L., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 62.

3 Cameron, G. R., and Karunaratne, W. A. E., *J. Path. and Bact.*, 1936, **42**, 1.

Histological examination revealed in these cases extensive hepatic necrosis with marked fatty infiltration and occasional small hemorrhage.

Allyl Formate. When injected into rats, allyl formate brings about typical hepatic lesions consisting of congestion of the sinusoids and hemorrhages which are visible within 1 or 2 hours after injection. Later, about 6 to 8 hours, there is periportal necrosis which is more pronounced in those animals that survive for 24 hours.[4] Mice were injected with allyl formate intraperitoneally, 0.0015 g dissolved in saline per 100 g of body weight. Of 21 mice, 4 died within 3 hours, 10 more died within 24 hours and the remaining 7 were killed or died between 24 and 30 hours. The histological picture was the same as described in rats, namely, sinusoidal congestion and hemorrhages at 3 hours, followed by vast periportal necrosis, visible to the naked eye, after 24 hours.

Salmonella enteritidis. Mice given by mouth a culture of *S. enteritidis* of low virulence, usually exhibited a mortality rate of about 20%, and did not regularly develop lesions in the liver. Mice injected intraperitoneally with a virulent strain, which in dilution of 10^{-6} was uniformly lethal in 5 or 6 days, showed in all instances mild lesions consisting of a slight congestion of sinusoids and edema within 2 or 3 days. Three or 4 days later, when sick or prostrate, these mice had advanced hepatic damage with focal necrosis, polynuclear and mononuclear cell infiltration both diffuse and in abscess-like collections, and marked edema.

Mouse-liver Autolysate. Injection into animals of extracts of organs derived from homologous species induce definite lesions.[5-7] By injecting mice with normal mouse-liver autolysate it has been possible to induce hepatic dysfunction and lesions. A group of 40 mice was injected as follows: a 10% normal mouse-liver suspension in saline was made up and merthiolate in final concentration of 1:10,000 was added. The suspension was kept at 37°C for 24 hours, then at room temperature for about 40 days. Beginning on the 5th day, mice were injected intraperitoneally with 1 cc. Similar injections were repeated every 3 or 4 days until a total of 10 injections was given. No mouse became ill or died during the course of the experiment. One month after the last treatment, 10 mice were killed and their livers were examined. Four showed gross changes, consisting of reduction in size of the liver, which had blunt, rounded edges, and a somewhat irregular surface with scar-like retractions. On microscopic examination these livers showed thickening of Glisson's capsule, increased periportal connective tissue, 1 or 2 cysts, focal necrosis and cellular infiltration. Surviving mice are still being observed.

Starvation. When mice were starved, death occurred in some on the 5th day, in most on the 6th and 7th days and one single mouse, of a total of 30, survived at least 8 days. Two mice were sacrificed daily and sections of the liver were made. In the first 3 days of starvation a mild fatty infiltration was noticed. After that some of the livers showed an increased fatty infiltration with cells harboring large fat droplets or, more often, the amount of fat receded and necrotic lesions about the central vessels appeared. The amount of necrosis was increased after 7-8 days; this necrosis affected first only the cytoplasm, later the nuclei.

Tests of Hepatic Dysfunction. In this section will be described the tests of hepatic dysfunction and their results, and the correlation of the latter with the degree of hepatic damage.

Thymol-turbidity Test. The method followed that of Maclagan[8] except that 0.1 instead of 0.05 cc of serum was used. The resulting opalescence was read in a Coleman spectrophotometer at lambda = 650 mμ and was expressed in terms of turbidity units by multiplying the optical density reading by 100. The units thus calculated agreed fair-

[4] Rosin, A., and Doljansky, L., *Am. J. Path.*, 1946, **22**, 317.

[5] Rivers, T. M., Sprunt, D. H., and Berry, G. P., *J. Exp. Med.*, 1933, **58**, 39.

[6] Morgan, I. M., *J. Bact.*, 1946, **51**, 53.

[7] Oettel, H., *Z. klin. Med.*, 1942, **141**, 773.

[8] Maclagan, N. F., *Brit. J. Exp. Path.*, 1944, **25**, 234.

ly closely within the range of values encountered with the units obtained from a standard curve derived from known amounts of bovine albumin precipitated with sulfosalicylic acid.

For this, as well as for the other tests, mice were bled from the heart under ether anesthesia. Only sera showing no or slight hemolysis were used. In general, mice were bled after withholding food overnight, since fed mice gave somewhat higher values, due probably to the presence of fat in the serum. Normal mice, not older than 8 months, and mice injected with a variety of materials such as 1 or 2 injections of mouse-liver suspensions, human serum or feces, gave readings ranging from 0.5 to 4 units. Of 275 sera from 150 mice, only 4 showed readings of 4 or 5 units. The over-all average for these sera was 1.7 units, and the averages for 25 separate groups varied from 1.5 to 2.6 units each.

Normal untreated mice, 14 and 18 months o'd, exhibited decidedly higher values; 16 such mice had an average of 5.5, and 9 of 16 showed 5 units or more (up to 15.1). Sections of the liver from 3 mice in this group revealed certain inclusion-body lesions as already described.[9]

Mice given phosphorus, carbon tetrachloride or liver autolysate, tested at the time when histological changes were found and when bilirubin or bromsulphalein-retention tests were positive in many, rarely exhibited thymol-turbidity higher than 4 units, the highest being 6.1. Of 120 sera tested, only 6 were higher than 4, with an over-all average of 2.1 units.

Mice inoculated with serum or feces deriving from human cases of infectious hepatitis, and then tested at different intervals after injection, likewise offered no indication of any rise in thymol-turbidity units. Two sera showed 6.9 and 5.2 units; the average value of 107 sera being 2.1 units.

Thus, the thymol-turbidity test in mice reveals that with 0.1 cc serum, an average of 1.7 units was obtained in normals or in animals injected with normal human sera or feces, or mouse-liver extract. Most of the individual readings were between 1 and 3 units.

In view of the results obtained with carbon tetrachloride, phosphorus, or human material derived from infectious hepatitis cases, the thymol turbidity test appeared to be valueless for the detection of hepatic changes induced in mice by these materials.

Plasma-coagulation Time. The coagulation time, as determined by the method of Howell, of recalcified plasma from normal mice was found to be from 1.5 to 4 minutes. Recalcified plasma from mice with hepatic lesions induced by carbon tetrachloride had a similar coagulation time.

Vaginal-smear Cytology. Studies of the estrous cycle were made, observing the cytology of vaginal smears in mice,[10] both normal and those with lesions caused by carbon tetrachloride. It was expected that if estrogen were kept in the blood at a continued high level as a consequence of damage produced in the liver by carbon tetrachloride, it might result in a prolongation of the estrus stage, detectable by the type of cells in the vaginal secretions. Daily observations for 10 to 15 days failed to show any difference between mice given carbon tetrachloride and controls. The estrous cycle was not affected, therefore, by extensive hepatic damage caused by poisoning with carbon tetrachloride.

Cephalin-cholesterol Flocculation Test. This test as described by Hanger,[11] when applied to normal mouse sera gave positive reactions in all cases. The degree of reaction was the same as that shown by mice with extensive hepatic lesions; therefore, it is not useful for the detection of such damage. Positive cephalin-cholesterol flocculation by normal sera has been previously described in several other animal species.[12]

9 Olitsky, P. K., and Casals, J., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 48.

10 Snell, G. D., editor, *Biology of the Laboratory Mouse*, by the Staff of the Roscoe B. Jackson Memorial Laboratory, Blakiston Company, Philadelphia, 1941, pp. 65-71.

11 Hanger, F. M., *Tr. Assn. Am. Physn.*, 1938, **53**, 148.

12 Recant, L., Chargaff, E., and Hanger, F. M., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 245.

Quantitative Determination of Blood-bilirubin. The method of Evelyn and Malloy, conveniently modified, was used for determination of bilirubin. Sera (or plasma) from mice kept off feed overnight was diluted 6 or 8 times in distilled water. The reagents were added to the test tubes as follows: methyl alcohol, 1 cc; diazo reagent (or diazo blank), 0.2 cc; serum in dilution 1:6 (or 1:8), 0.8 cc. After standing for 30 minutes at room temperature, the color reaction was read in the Coleman spectrophotometer at lambda = 550 mμ. From the standard curve obtained with known amounts of bilirubin, the bilirubin content in mouse serum, in mg per 100 cc, was determined by multiplying the amount given by the meter reading by the dilution factor, 6 or 8.

In 66 normal mice the average amount of bilirubin in 100 cc of serum (or plasma) was 0.3 mg; most values (55 out of 66) were lower than 0.5 mg; the highest was 1.2 mg.

In 40 mice given phosphorus, subcutaneously or orally, the average amount of bilirubin was 0.4 mg per 100 cc of serum, with similar distribution to that of the controls, *i.e.,* 28 of 40 were lower than 0.5 mg. As described in the previous section, most of these mice failed to be affected by phosphorus. However, even in 6 or 8 mice, which were visibly ill and had hepatic lesions, the bilirubin in plasma was not high; the highest in these was 1.2 mg per 100 cc of plasma, and the average was about 0.6 mg.

In 33 mice injected with carbon tetrachloride and tested within 7 days, the average bilirubin in 100 cc of plasma was 0.6 mg; 24 of the 33 had 0.5 mg or higher. The highest reading encountered was 1.6 mg. When mice injected with carbon tetrachloride were tested after 8 to 26 days, the average in 12 mice was 0.3 mg per 100 cc, with 10 lower than 0.5 mg, the highest value was 0.7 mg.

These observations show that the amount of bilirubin in the plasma of mice with hepatic lesions induced by phosphorus or carbon tetrachloride, is somewhat higher than in control mice; however, this increase is neither constant nor pronounced.

Bromsulphalein-retention Test. Of the tests investigated, retention of bromsulphalein has been the most sensitive and accurate indicator of the lesions brought about in the liver by the means already described. In a few instances lesions existed without influencing the retention of bromsulphalein and not always did the degree of retention reflect the extent of the hepatic damage. In general, however, in mice both in groups or as individuals, bromsulphalein-retention was found to be a reliable test for detecting hepatic damage.

In order to develop a method applicable to mice a number of modifications were tried, involving the route of injection of the dye (intravenous, intraperitoneal, subcutaneous), amounts of dye and time of bleeding. A method was finally evolved which is practical and simple even for large numbers of mice. It yielded the largest number of positive results in the presence of hepatic lesions, was practically free from false positives, and within limits, permitted the testing of mice repeatedly with a minimum of deaths.

Method. Mice are weighed individually to the nearest gram. Bromsulphalein is injected intraperitoneally, 0.05 mg in 0.05 cc physiological saline per g of body weight. Thirty minutes later the mouse is bled from the heart; 0.5 cc of blood is deposited in a 6 mm diameter tube containing 0.05 cc of a 2% solution of potassium or sodium oxalate in saline. The blood is centrifuged at 1500 r.p.m. for 15 minutes and the plasma is removed with a capillary pipette. As a rule, 0.22 to 0.25 cc of plasma is obtained; 0.1 cc of plasma is placed in each of 2 Coleman cuvettes (N° 6-308C) and 1.5 cc of physiological saline is added to each, a dilution of 1:16 of the plasma. Then, to the first cuvette is added 0.05 cc of a 10% solution of sodium hydroxide, to the second 0.05 cc of a 10% dilution of hydrochloric acid. A control, consisting of a known amount of bromsulphalein in saline solution is included in each test. The colorimetric reaction is read in the spectrophotometer at lambda = 580 mμ. The values for light transmission are compared with those shown in the standard curve obtained with known amounts of

TABLE I.
Bromsulphalein-retention in Mice Injected with Carbon Tetrachloride.

Days after injection	No. of tests	Bromsulphalein in plasma in mg per 100 cc			% of individual-values higher than 1 mg
		Avg	Highest	Lowest	
1	27	8.4	27.2	2.3	100
2	22	10.6	21.4	2.1	100
3	5	4.1	10.9	.3	60
4	23	2.6	10.1	.4	83
6	6	2.4	6.2	.4	50
8	16	.4	1.1	.2	6
9	11	.9	1.7	.3	27
11	10	.3	.45	.2	0
16	12	.3	.45	.1	0
27	7	.6	1.0	.3	14
31	7	.4	.6	.3	0
Controls, no carbon tetrachloride	346	.46	2.0	.1	4.6

bromsulphalein, read in mg per 100 cc of plasma. Since the plasma is diluted 16 times one must multiply this amount by 16, thus obtaining the amount of bromsulphalein in undiluted plasma. By staggering the injection of dye and bleeding, it is possible to test 20 to 30 mice in about 2 hours. The result of the test is not expressed as the percentage of bromsulphalein-retention as is done in human tests, but rather as the amount of bromsulphalein in plasma, in mg per 100 cc. It should be stated here that in this test (as well as in the determination of bilirubin) the amount of dye in mg per 100 cc of plasma must be considered only as an approximation, hence these tests are not to be taken as exact biochemical determinations. Furthermore, the readings are made in a region where the instrumental error is highest, and the necessity of diluting the plasma introduces still another source of error. However, within these limitations the test has proved workable and dependable in mice and since it has been more sensitive than the other tests here described, it was used extensively. The intraperitoneal injection of dye was used in preference to the intravenous because the former is more practical and is equally sensitive: the absorption of bromsulphalein from the peritoneal cavity began immediately and lasted from 2 to 18 hours. This was determined by titrating the amount of dye left in the peritoneal cavity washed with saline. The elimination of dye began in less than 5 minutes, after injection, as was shown by its recovery from the intestinal contents. A balance is probably struck between the rate of absorption from the peritoneal cavity and the rate of elimination by the liver, which results in a shifting level of bromsulphalein in the blood. There have been no visible ill effects resulting from the injection of this relatively large amount of dye in mice. Moreover, when the same mice were tested 2 or 3 times in succession at 5- or 6-day intervals, there was no indication of an increased retention in the 2nd or 3rd test due to cumulative effects.

TABLE II.
Bromsulphalein-retention in Mice Given *Salmonella enteritidis* of Low Virulence.

Days after injection	No. of tests	Bromsulphalein in plasma in mg per 100 cc			% of individual values higher than 1 mg
		Avg	Highest	Lowest	
4	18	.55	1.2	.1	5.5
9	23	.67	2.0	.1	17.4
14	12	1.0	1.8	.3	41.7
21	6	.9	1.7	.4	33.0

TABLE III.
Bromsulphalein-retention in Mice Given *Salmonella enteritidis* of High Virulence.

Method of dye test	Treatment	When tested	No. of mice	Modified methods: Bromsulphalein in plasma in mg per 100 cc — Avg	Highest	Lowest	% of individual values higher than 2 mg
0.1 mg subcutaneously; bled at 40 min.	Injected	3rd day	8	1.3	3.1	.5	12.0
	Controls		16	1.0	2.3	.4	6.0
0.1 mg intraperitoneally bled at 30 min.	Injected	4th-5th day	14	3.65	5.8	1.7	78.5
	Controls		15	1.75	3.7	.7	26.5

TABLE IV.
Bromsulphalein-retention in Starved Mice.

Days after starvation began	No. of mice	Bromsulphalein in plasma in mg per 100 cc — Avg	Highest	Lowest	% of individual values higher than 1 mg
None (control)	24	.53	1.0	.1	4.0
1	26	.88	2.4	.3	26.9
2	26	.70	2.2	.1	26.9
3	24	.90	2.4	.1	45.8
4	20	1.18	2.3	.3	60.0
5	10	1.74	2.5	.2	80.0
6	4	4.2	8.3	2.0	100.0
7	3	2.4	2.6	2.2	100.0
8	1		8.4		100.0

Results. In 138 normal mice the average amount of bromsulphalein in plasma at the end of 30 minutes was 0.47 mg per 100 cc; in 208 mice injected with human serum or feces, both from healthy individuals and from individuals with infectious hepatitis, or with fresh mouse-tissue suspensions, the amount of bromsulphalein averaged 0.45 mg per 100 cc of plasma. Since the results of the tests in these 2 groups were similar, they were pooled. Thus, there were a total of 346 mice with an average retention of 0.46 mg per 100 cc; 83% were lower than 0.7 mg; 63% were lower than 0.5 mg; 4.6% showed readings higher than 1 mg; the highest single value was 2 mg (Table I).

It is evident, therefore, that any individual value of bromsulphalein-retention lower than 1 mg per 100 cc is normal, between 1 and 2 mg per 100 cc may be considered as questionably, and higher than 2 mg as definitely increased.

Of 146 tests with mice injected with carbon tetrachloride (Table I) most of them exhibited within 6 days significantly increased bromsulphalein-retention. As stated in the previous section, this was the time in which lesions were found; after the 8th day neither positive dye-retention nor visible lesions occurred.

Of 14 mice given phosphorus either orally or parenterally, tested on the 5th day, there were 4 values higher than 1 mg of bromsulphalein per 100 cc of plasma, namely, 19.7, 5.7, 1.7 and 1.2. Allyl formate poisoning resulted in retention of bromsulphalein greater than that shown by controls. Of 8 mice tested 3 hours after injection, 3 had values higher than 2 mg per 100 cc and 4 between 1 and 2 mg; the lowest, 0.9 mg and the highest 8.6 mg, with an average of 2.6 mg per 100 cc. Mice receiving liver-autolysate exhibited, after the early injections, an average of 0.35 mg; after all the injections, 0.75 mg. It will be noted that significant differences occur between the bromsulphalein-retention of mice given *S.*

enteritidis and normal ones (Tables II and III).

Finally, mice starved from 1 to 8 days were tested for retention of the dye (Table IV). It is of interest that a mild increase in the retention of bromsulphalein arose even in the early days of starvation. Beginning with the 5th day, however, at a time when either marked fatty infiltration or necrotic lesions was observed histologically and mice had begun to die, the retention of bromsulphalein was decidedly increased, as shown by the fact that all but 2 of the mice tested on the 5th day or later had readings higher than 1 mg.

Summary. Six tests for the study of hepatic dysfunction and damage were applied to Swiss mice. Insufficiently sensitive to detect hepatic lesions were the thymol-turbidity test, plasma-coagulation time and vaginal-smear cytology. Cephalin-cholesterol flocculation was not applicable owing to the fact that normal mouse sera reacted positively. Blood-bilirubin determinations indicated hepatic lesions induced by carbon tetrachloride or phosphorus. The increase in bilirubin in the blood, however, even in cases of extensive damage, was not high; 0.3 mg per 100 cc of plasma in control mice as an average, as compared with 0.6 mg per 100 cc in those with carbon tetrachloride lesions.

Retention of bromsulphalein was the most useful and accurate of all the tests described herein. A base line was established for control mice; *i.e.*, less than 1 mg of dye retained per 100 cc of plasma could be regarded as normal. In mice with hepatic lesions, tested at a proper time and within the limitations mentioned, values 10 or 20 times higher than the normal were not unusual. Since retention of this dye was increased by diverse methods such as chemical poisoning, bacterial infection, starvation, and injection of autolyzed tissue, its use may be of value in the study of experimental problems connected with hepatic injury of mice.

15610

Permanent Ablation of Bone Marrow in Long Bones of Living Rabbits.

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In a previous paper a method of removing marrow from a single bone in living rabbits was described.[1] In this article a procedure is outlined in which the marrow of all the long bones was removed and replaced by inert material. The regeneration of marrow was prevented permanently in the bones so treated. On the basis of Dietz's analyses of distribution of bone marrow,[2] it was estimated that approximately 41 ± 5% of the total marrow may be ablated by this method.

The rabbit was the animal of choice. The marrow in this animal is tubular and with very few connective tissue bands which bind it to the endosteum. The complete marrow is therefore readily expressed. The animal is also sufficiently large to withstand several operative procedures. The marrow was removed from 2 to 10 bones: both tibiae, both femora, both humeri, and both radii and ulnae. The marrow of all other bones is inaccessible by this method. Thirty rabbits were used in the experiment over a period of 3 years.

The animals were anesthetized with pentobarbital sodium. The initial steps in drilling holes in the bone with a Ralk nail drill and expressing the marrow were similar to those already described.[1] Cotton strips of varying lengths and thickness were saturated in bone wax and autoclaved in Petri dishes. The

1 Steinberg, B., and Martin, R. A., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 428.

2 Dietz, A., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 60.