TABLE I. No. of Ossified Sternebrae in Fetuses of Control and Insulin-Treated Rats with 22 Days Gestation.

Stained sternebrae	28 litters —Control— No. of fetuses	%	11 litters —Insulin, 7 units/day— No. of fetuses	%	29 litters —Insulin, 8 units/day— No. of fetuses	%	
6	83	98.8	50	94.3	66	51.16	
5	1	1.2	0	0	10	7.75	66.66
4	0	0	0	0	10	7.75	
3	0	0	2	3.8	13	10.08	
2	0	0	0	0	11	8.53	33.34
1	0	0	0	0	10	7.75	
0	0	0	1	1.9	9	6.98	
Total	84		53		129		

this possibility must of course be considered in the interpretation of results such as those reported here. Duraiswami(9) found, however, that inactivation of insulin by the methyl-alcohol-hydrochloric acid method of Charles and Scott rendered it ineffective in producing abnormalities in developing chicks; in his experiments the effect of insulin on developing chick embryos appeared to be linked with its blood-sugar lowering action. After noting that the skeletal abnormalities induced by insulin in chicks resembled the abnormalities produced in rats by maternal diets deficient in riboflavin, as reported by Warkany and coworkers(1-3), Duraiswami speculated on the possibility that insulin induced hypoglycemia might involve disturbances in phosphorylating enzyme systems such as arise with a lack of vit. B complex, thus causing disturbances of carbohydrate metabolism in embryonic tissue during critical periods of development. Giroud's studies(15,16) on the effects of avitaminosis-B_2 on embryonic development in the rat also are pertinent to such speculations.

Summary. Protamine zinc insulin administered to 51 pregnant albino rats throughout pregnancy in doses 7 and 8 units daily (nearly the maximum dose tolerated) had significant effects on fetal development. Compared with fetuses of untreated normal control rats, the average weight of the fetuses of the insulin-treated rats was lower, and the number in each litter was less owing to fetal deaths and resorption. Mild skeletal abnormalities were found, many of the fetuses showing a decreased number or complete absence of ossification centers in the sternum, a few showing some irregularities in shape and staining of the ribs and long bones.

15. Giroud, A., and Boisselot, *J. Arch. Franc. pédiat.*, 1947, v4, 317.

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Received July 23, 1951. P.S.E.B.M., 1951, v78.

Effect of Protein Depletion on Rate of Disappearance of Radioactive Protein from the Blood Stream. (19085)

CHARLES A. HUBAY, HARVEY KRIEGER, WILLIAM D. HOLDEN, AND THOMAS HANCOCK.

From the Department of Surgical Research, Western Reserve University School of Medicine and the Department of Surgery, University Hospitals of Cleveland.

The relationship of the effective circulating blood volume and disease states has assumed an important place in surgical physiology. Several methods of determining the blood volume have been advanced, including the use of radioactive (I-131) iodinated plasma

protein. Storaasli(1) and his co-workers demonstrated that the stability of the iodinated protein and its relatively secure retention within the vascular system permits a more accurate estimate of the plasma volume than has hitherto been possible. Previous work (1-5) has shown that after radioactive iodinated plasma protein is injected intravenously, there is a progressive decrease in the measurable radioactivity of blood samples withdrawn periodically. Storaasli, *et al.*(1) showed that the method of disappearance of iodinated protein from the vascular system is two fold: (a) by the diffusion of the protein into extra vascular spaces shown by studies of thoracic duct lymph(3,4) and (b) by liberation of the radioactive iodine from the protein as manifested by excretion of iodine in the urine and increased radioactivity over the thyroid gland.

This method of blood volume determination was shown to be reasonably accurate in normal individuals and we studied its use in pathophysiological states. The work reported here was done in experimental animals and human patients with hypoproteinemia. The existence of endogenous reserve stores of protein has been demonstrated by Whipple(6). When these reserve stores are depleted by inadequate diet, excessive loss, or metabolic dysfunction, the ability of tissues to withstand trauma and to repair themselves is decreased. It was thought worthwhile to investigate the rate of disappearance of tagged protein from the blood stream to see whether or not the state of the protein reserves of the body could be determined by deviations from the normal disappearance rate. This has great clinical importance, particularly in surgery, in determining the optimum time for operation in malnourished and debilitated patients.

Method. The disappearance rates (Fig. 1) of tagged protein were determined in normal healthy mongrel dogs, on standard kennel diets. These dogs were then placed on a low protein diet, similar to the one employed in G. H. Whipple's laboratory(7). Nitrogen balance studies were not feasible but the amount of nitrogen per day in the diet did not exceed one gram. Eleven dogs were studied but several animals refused to eat this low protein diet and depletion studies were possible in only 5 animals. After 4 to 8 weeks on this low protein diet disappearance rates of radioactive protein were again determined. During this time the plasma protein levels and body weights were determined weekly. Following these periods of protein deprivation, an attempt was made to replete the animals to a normal nutritional state by placing them on the standard kennel diet, but all died of intercurrent infection or distemper, except animal No. PD 5 which recovered. The radioactive (I-131) protein* was prepared from dog plasma by a modification of the method of Fine and Seligman(8). Preparations are now available utilizing human serum albumin for study of human patients.† The technic used in determining the plasma volume involves the measurement of the dilution of tagged protein after intravenous injection and has been described(1,2). Twenty cubic centimeters of the tagged protein solution were injected intravenously and periodic venous samples were withdrawn without stasis at 10, 15, 30 minutes, 1, 2, 4, 6, 8, 12 and 24 hour periods. The blood was placed in tubes containing crystalline heparin and then centrifuged at 2500 RPM for 30 minutes. The

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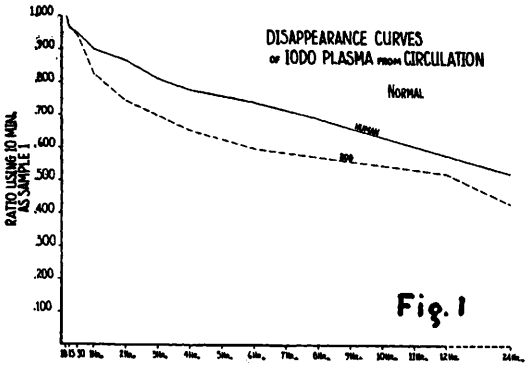
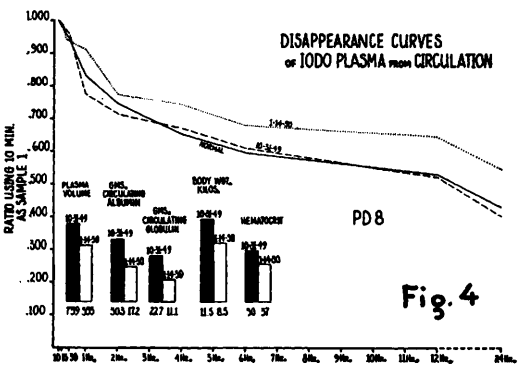
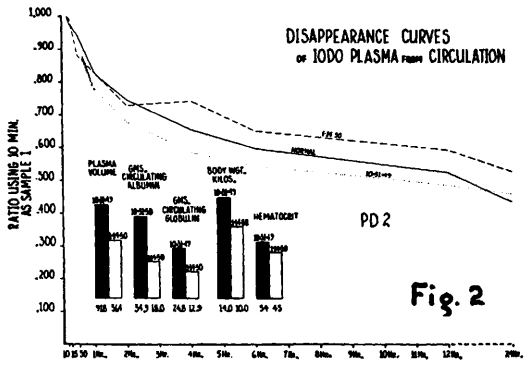
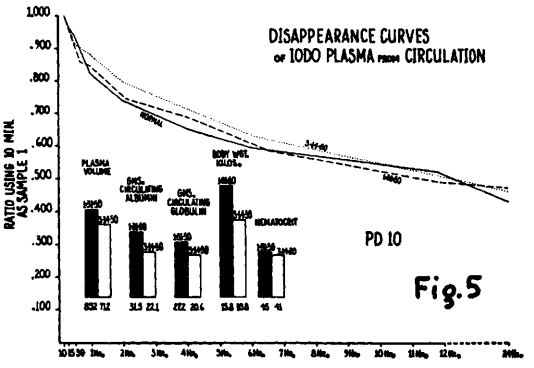
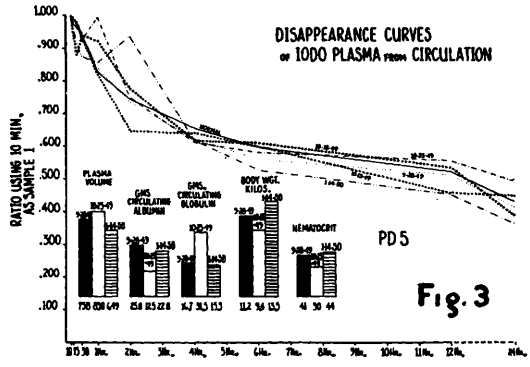
† The Abbott Laboratories generously supplied us with the radioactive albumin used in human cases in this study.











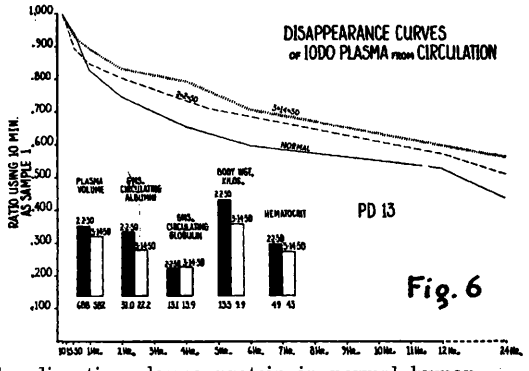


FIG. 1. Average rates of disappearance of radioactive plasma protein in normal human patients and dogs. Reproduced in part from Storaasli *et al., Surg. Gyn. & Obst.*, Oct., 1950, by permission of the publishers.

FIG. 2. Disappearance curves of radioactive plasma protein from circulation of dog PD 2; basal condition 10-31-49, protein depletion, 1-14-50.

FIG. 3. Disappearance curves of radioactive plasma protein from circulation of dog PD 5; basal condition 9-28-49; protein depletion 10-25-49; protein repletion 1-14-50.

FIG. 4. Disappearance curves of radioactive plasma protein from circulation of dog PD 8; basal condition 10-31-49; protein depletion 1-14-50.

FIG. 5. Disappearance curves of radioactive plasma protein from circulation of dog PD 10; basal condition 2-2-50; protein depletion 3-14-50.

FIG. 6. Disappearance curves of radioactive plasma protein from circulation of dog PD 13; basal condition 2-2-50; protein depletion 3-14-50.

supernatant plasma was removed and 1 cc samples were then counted by means of a Geiger-Muller tube and the activity determined. Total protein, A/G ratios were determined by the Kingsley(9) method utiliz-

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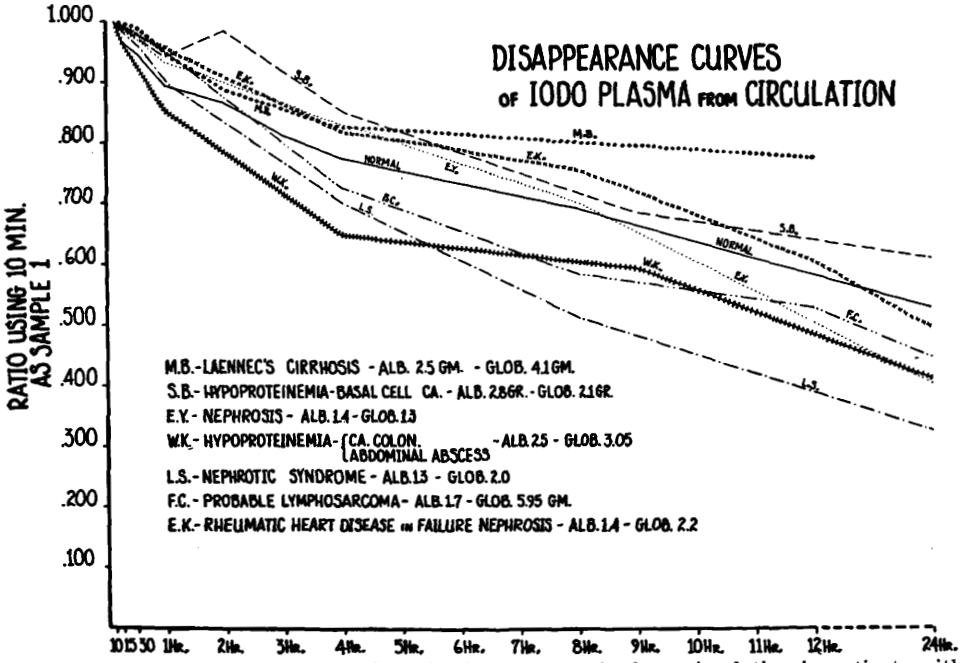

FIG. 7. Disappearance curves of radioactive human albumin from circulation in patients with hypoproteinemia. Expected normal rate of disappearance is shown.

ing the Klett-Summerson colorimeter. Disappearance rates were calculated as activity per cc of plasma and expressed as a ratio using the 10-minute sample as 1.000.

Results. The average normal rate of disappearance of radioactive plasma protein in 11 normal dogs is shown in Fig. 1. This rate of disappearance correlates closely with that described by Fine and Seligman(8) and Cope and Moore(4). Fig. 2 to 6 represent the graphic rates of disappearance of each dog studied with weight loss and hypoproteinemia. In general the differences tend to be an average of 6% slower in the depleted state, and we have shown that at least in one dog (PD 5) this decreased rate of disappearance in the hypoproteinemic state was reversed when the dog was repleted to a normal nutritional state.

On the basis of this study, we do not believe that this diminution in rate of disappearance in the hypoproteinemic state is significant. We feel justified in concluding that the rate of disappearance of radioactive plasma protein cannot be utilized in estimating the state of the protein reserves of the tissues in the dog. Metcalf(10) studied dogs placed on protein free diets and then plasmapheresed them to near edema levels. He found that transfused serum disappeared in protein depleted animals in quantities of the same magnitude as in control animals, and that after protein repletion, the disappearance curves were essentially unchanged.

The plasma volumes and disappearance rates in normal individuals were studied in 30 patients by Storaasli(1). The average disappearance curve is shown in Fig. 1. Seven patients with hypoproteinemia were studied and disappearance curves calculated after injection with tagged (I-131) human serum albumin (Fig. 7). In these patients the rate of disappearance of radioactive iodinated albumin parallels the average normal curve, and the differences seen are probably due to variation in fluid intake, ambulation, etc. In the patients who are losing abnormal amounts of protein in the urine (E. Y., L. S., E. K.) or into abscess cavities (W. K.), the rate of disappearance appears to be increased. In the patients with nephrosis, there is an in-

10. Metcalf, Wm., *J. Clin. Invest.*, 1944, v23, 403.

creased amount of radioactive iodine recoverable from the urine in a 24-hour period (38%) over that found in the urine of the normal patient (8-12%).

Summary. 1. Plasma volumes can be accurately determined by the radioactive (I-131) iodinated plasma protein method in nutritional hypoproteinemia. 2. The rate of disappearance of radioactive protein from the blood stream is the same in undernourished states as in normal and therefore cannot be utilized to determine the state of the protein reserves of the body. (3). In patients who are losing abnormal amounts of protein in the urine or into abscess cavities, the rate of disappearance appears to be increased. The use of radioactive serum albumin may offer a diagnostic aid in the detection of unexplained protein losses.

The authors gratefully acknowledge the technical assistance of Miss Jean Hower and Mrs. Eileen Davis and wish to thank Doctors Ole Jonassen and Frank Cebul for their aid in the completion of some of these studies.

Received July 26, 1951. P.S.E.B.M., 1951, v78.

In vivo Staining of Fat in Tumor Bearing Mice by Benzo[a]phenoxazine Dyes.* (19086)

MARGARET REED LEWIS, M. L. CROSSLEY, AND P. F. DREISBACH.

From the Wistar Institute of Anatomy and Biology, Philadelphia, Pa., and the Calco Chemical Division, American Cyanamid Co., Bound Brook, N. J.

In a previous study(1) of the effects of the oral administration of dyes on tumor bearing mice it was found that certain benzo[a]phenoxazines containing 5-phenylamino groups stained fat *in vivo*. The N-phenyl derivative of Nile Blue A stained fat a brilliant orange and tumor tissue a pale blue color. On the other hand, it was shown that the 5-amino and 5-benzylamino - 9 - diethylaminobenzophenoxazines, Nile Blue A and Nile Blue 2B, respectively, stained tumor tissue deep blue but failed to stain fat *in vivo*. The failure of the 5-amino and the 5-benzylamino-9-diethylaminobenzophenoxazines to stain fat *in vivo* was of interest because Thorpe(2), Smith(3), and Heidenhain(4) had shown that these compounds stain fat *in vitro*. The *in vitro* differential fat staining effect was supposed to depend to some extent upon the fat solubility of the dyes or their chemical transformation products. Hadjioloff(5), however, in his review of the publications on *in vivo* staining of fat administered by the enteric route, showed that many fat soluble dyes failed to stain fat *in vivo*. In another study of the benzo[a]-phenoxazine dyes(6) it was found that of the dyes available at that time only those containing the 5-phenylamino group stained fat *in vivo*.

The present study is concerned with *in vivo* staining of fat in tumor bearing mice following oral administration of a number of substituted 5,9-diaminobenzo[a]phenoxazine dyes. The majority of these contained a 5-phenylamino group while the others had benzyl-, naphthyl- or heterocyclic amino groups in the 5-position. Generally, the hydrogens of the 9-amino group were replaced by alkyl radicals containing from one to 6 carbon atoms.

Material and method. Mice of inbred stains and tumors which were 100% transplantable in mice of the strain of the host

* Aided in part by a grant to Dr. Margaret Reed Lewis from the National Cancer Institute.

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