lated. Presence of secretion in seminal vesicles of Group 17 only, indicated that higher priming dose of TP was required before LTH could cause it to become secretory. Doubling the dose of LTH to 60 I.U. daily (Group 18) caused corresponding increase in weight of seminal vesicles. Since there is no increase in height of epithelium the increase in weight could be accounted for primarily by increase in amount of secretion or by hyperplasia caused by LTH.

The difference in appearance of seminal vesicle epithelium between autografted intacts (Group 12) and autografted castrates (Group 13) may indicate that there was a small amount of secretion of gonadotrophic hormones by transplanted pituitary which stimulated the Leydig cells to produce testosterone. This augmented the subminimal TP injected exogenously. It is also possible that the epithelial response by autografted intact animals may indicate that small amounts of LTH were being produced by the graft. This acted synergistically with subminimal TP to bring about responses similar to those seen in animals receiving subminimal TP and a high dose of LTH.

When comparing groups 16 and 17, there seemed to be no significant change in seminal vesicle morphology resulting from daily administration of 30 I.U. LTH over that resulting from TP itself. Contrasting these 2 groups with Group 18 which received 60 I.U. LTH, it appeared as if some stimulation was obtained other than by TP. Thirty I.U. LTH acting with subminimal dose of TP in the long term study groups did show stimulation. It is clear that many levels of LTH must be tested, however, some quantitative relationships of effectiveness have been suggested.

Summary and conclusions. The role of LTH (luteotrophic hormone, prolactin or lactogenic hormone) in stimulating epithelium of seminal vesicles of castrated guinea pigs has been tested in 2 series of experiments. LTH alone had no effect on seminal vesicles of castrates. LTH together with subminimal testosterone propionate caused significant increase in weight of seminal vesicles as well as height of epithelium. A high priming dose of TP was required before LTH stimulated visible secretion. Doubling the dose of LTH after priming with TP caused a corresponding increase in seminal vesicle weight. Prostates, testes, mammary glands, adrenals and pituitaries did not show any significant differences in structure or size when various levels of LTH were present. The results are in agreement with the findings of Pasqualini(1) and Chase *et al.*(2).

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Distribution of Red Blood Cells Between Tissues of Mouse.* (25419)

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Red blood cells labeled with radioactive isotopes are presently being employed in a variety of experimental conditions for determination of circulating red blood cell volume. In general, the technic consists of intravenous injection of labeled erythrocytes followed by sampling of blood after a period of time considered adequate for complete vascular mixing. Usually, this time interval is derived from the blood radioactivity curve and represents the point at which blood radioactivity achieves a constant level. While this interval may be suitable for determination of the cir-

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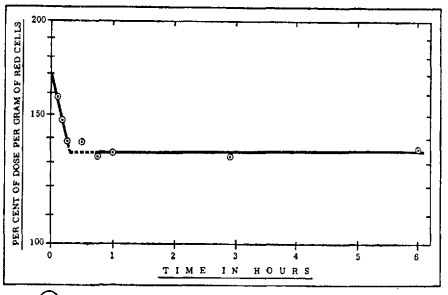

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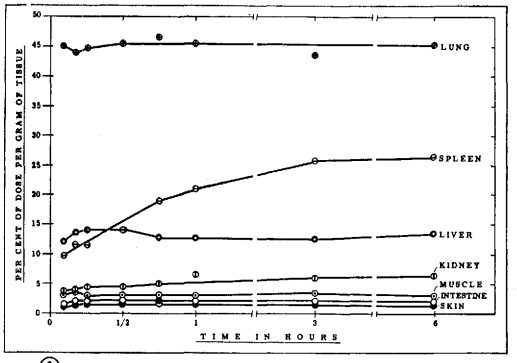

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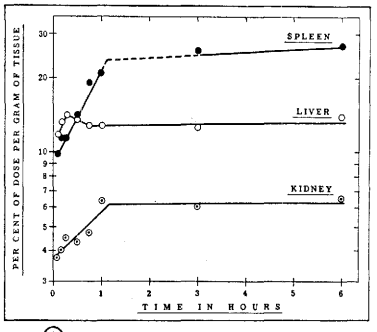

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FIG. 1. Concentration of radioactivity in circulation following intrav. administration of radioiron labeled red cells (semi-log plot).

FIG. 2. Distribution of intravenously inj. radioiron labeled red cells between various tissues of mouse.

FIG. 3. Distribution of radioactive red cells between liver, kidney and spleen following intrav. administration (semi-log plot).

culating red cell volume it does not necessarily indicate that vascular mixing has been completed at the tissue level. It is certainly conceivable that incomplete vascular mixing may exist in some segment of tissue vasculature and be obscured by the large pool of radioactivity in the effective circulation. Because it was of interest to study tissue circulation, a knowledge of the characteristics of local vascular mixing was considered essential. Therefore, the progressive accumulation of radioactivity in various tissues following intravenous injection of labeled red cells was determined.

Methods. Ninety male albino mice of the CF-1 strain weighing 19-22 g were used. Mouse red cells were labeled *in vivo* with radioactive iron (Fe-59) according to the method of Hahn *et al.*(1). Essentially the procedure consisted of intravenous injection of a donor animal with radioactive ferrous ammonium citrate, followed by removal of blood from donor animal at approximately 3 weeks post injection. Three weeks of *in vivo* incubation resulted in about 50% incorporation of radioiron into the red cells. The cells were washed 3 times with cold saline, then reconstituted to hematocrit of 45%, previously determined to be the mean venous hematocrit of normal mice. The radioactive cell suspension was injected intravenously in volumes of 0.1 ml. The mice were then killed by rapid immersion into liquid nitrogen at intervals of 5 minutes to 6 hours post injection of tracer cells. Tissue samples were obtained(2), weighed, digested in alkali, and assayed for radioactivity with a scintillation counting system. Radioactivity in each tissue was expressed as per cent of injected dose of radioactivity/g of tissues.

Results. Fig. 1 represents a semi-log plot of radioactivity in the circulating blood, following intravenous injection of labeled red cells, expressed as per cent of injected dose/g of red blood cells. The point at each interval represents the mean of 10-12 determinations. Radioactivity/g of red cells exceeds the dose injected because the circultaing red cell volume is less than 1 g, thus when considered on the basis of 1 g, radioactivity associated with red cells is expanded proportionately. The dilution of vascular mixing of injected cells is indicated by an abrupt decline in concentration of blood radioactivity. This decline proceeds in a uniform manner for approximately 15 minutes, at which time blood radioactivity achieves a steady level, essentially maintained through 6-hour period of analysis. The point of transition is taken to represent the time at which vascular mixing of injected cells becomes complete. Fifteen minutes for

vascular mixing of red cells in the mouse agrees quite well with times reported for the dog(3) and human(4). It was expected that because of its accelerated heart rate and shorter circulation time, the mouse would exhibit a more rapid vascular mixing than either dog or human. This apparently prolonged mixing time of 15 minutes may reflect the intravenous injection of a relatively large volume of blood (5% of the blood volume) which would probably require additional time to become fractionated and completely mixed with the endogenous circulating blood.

Distribution of radioactive red cells throughout the tissue circulation is illustrated in Fig. 2. Tissue radioactivity is expressed as per cent of injected dose/g of tissue. The points at each interval represent the means of data derived from 10-12 mice. Since tissues such as lung and spleen are present in small amounts, when expresed on a per gram basis, the value of their radioactivity is expanded proportionately. On the other hand, radioactivity of tissues such as muscle, skin, and intestine which are present in the animal in quantities greater than 1 g, are attenuated when considered on a per gram basis. It will be noted that although concentration of radioactivity in skin, intestine, muscle and lung has reached a steady level within 15 minutes, that in the spleen, kidney and liver has not. To visualize more clearly the characteristics of radioactive accumulation in these tissues, the data presented in Fig. 2 were replotted on a semi-log scale and are shown in Fig. 3. Spleen and kidney exhibit a progressive increase in radioactivity for approximately 1 hour at which time very little further change occurs. During the first hour post injection of radioactive red cells, radioactivity within the liver first rises to a peak and then proceeds to decline. The initial increase in radioactivity of spleen and kidney is considered to represent rate of vascular mixing in these tissues. While these rates may seem slow, it is conceivable that becaue of the sinusoidal nature of the splenic vasculature the distribution of red cells throughout the spleen is somewhat restricted and irregular(5). The variable changes in liver radioactivity following intravenous injection of radioactive cells may also reflect the irregular and periodic flow of blood through the sinusoids of the liver(6). It is possible that a slug of concentrated radioactivity may have become momentarily isolated within inactive sinusoids of the liver thereby imparting a high tissue radioactivity to the organ. Subsequent activation of such sinusoids would permit the concentrated radioactive cells to be flushed out and liver radioactivity would decline. While the kidney does not possess a sinusoidal vasculature it appears to have the ability to restrict distribution of red cells throughout its circulation. This is consistent with the low dynamic hematocrit exhibited by the kidney of the mouse(7).

It is, of course, assumed that all injected radioactivity is securely bound in red cells and remains there so long as the cells maintain their integrity. That concentration of radioactivity in the blood remains essentially constant after the 15-minute post injection period suggests that either this assumption is valid or that any loosely bound radioiron in the injection suspension has been removed from the circulation with 15 minutes post injection.

It appears from our data that 15 minutes post-injection, required for mixing of labeled red cells with the rapidly circulating red cell pool, is also adequate for vascular mixing within skin, intestine, muscle and lung. However, it does not provide sufficient time for complete vascular mixing of red cells within spleen, kidney and liver. In these tissues, approximately 45 minutes to 1 hour is necessary to insure complete mixing of radioactive and non-radioactive red cells.

Summary. Distribution of intravenously injected radioactive red cells throughout tissue circulation of mice was determined. The evidence suggests that distribution of labeled red cells throughout the circulation of spleen, kidney and liver proceeds more slowly than in skin, intestine, muscle and lungs.

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Enhancement of Experimental Atherosclerosis by ACTH in the Dog.* (25420)

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Prolonged treatment with large doses of ACTH or cortisone is known to elicit a hyperlipemic response in the rabbit(1-3) in man (4), and to a lesser extent in the dog(5). Cholesterol-induced hyperlipemia in rabbits and dogs is heightened markedly during administration of ACTH, cortisone, or hydrocortisone (5-7). The present studies show that prior short-term administration of ACTH enhances development of hyperlipemia in dogs subsequently given a standard thiouracil and cholesterol diet for 6 to 10 months.

Methods. Atherogenic diet: The standard diet consisted of 2 daily doses of 50 mg thiouracil/k of body weight and kibble containing 10% cholesterol (prepared with ether solvent), to provide a daily cholesterol intake of 750 mg/k of body weight(8). To insure complete intake, this diet was fed first, followed by a meat-kibble diet fed *ad lib*. Dogs were divided into 3 groups: *Group A*. Twenty-eight dogs (13 beagles, 3 "Kendall mongrels,"† and 12 ordinary mongrels) were normal controls throughout and fed a meat-kibble diet only. *Group B*. Fourteen dogs (8 beagles and 6 "Kendall mongrels") were maintained on standard atherogenic diet, plus meat-kibble. *Group C*. Seven dogs (4 beagles, one "Kendall mongrel," and 2 mongrels) were given one to 3 injections of 40 units of ACTH-gel subcutaneously at intervals of 6 to 12 days. Two to 4 months later, the 7 dogs were placed on the atherogenic diet. Three additional male beagles received 3 subcutaneous injections of 40 units ACTH-gel at 3 or 4 day intervals. After the third injection, the 3 dogs were started on the atherogenic diet. All dogs but the ordinary mongrels were approximately 1 year of age. All were maintained in kennels with outside runs. Each dog was used as his own control for at least one to 4 months. Each was weighed at 2-week intervals and fed the atherogenic diet individually on a body-weight basis. Blood samples drawn from fasting dogs at 2 to 4 week intervals were analyzed for serum cholesterol(9), phospholipids(10) and alpha and beta lipoproteins (11). At autopsy, tissues were fixed in 10% neutral formalin. Aortas were stained with Sudan IV to demonstrate lipid content of atheromatous lesions(12).

Results. Group A. Without treatment, serum lipid values of beagles and ordinary mongrels were comparable (Table I). Cholesterol and phospholipid values for the "Kendall mongrel" tended to be higher ($p = <0.05$‡) than in the normal beagle. C/P and lipoprotein ratios did not differ significantly between beagles and "Kendall mongrels."

Group B. The atherogenic diet fed 4 to 11 months tended to elevate serum cholesterol, phospholipids, and C/P ratio in all dogs, ($p = <0.05$) and a marked rise occurred in %

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† It has been suggested that breeds of dogs differ in susceptibility to atherosclerosis: beagles are considered to be relatively resistant while a strain, originally mongrels and inbred by Dr. Forrest Kendall, are relatively susceptible.

‡ The rank sum test was used for statistical evaluation of data.