sulting from hormone administration. Methods of analysis for a wide spectrum of metabolites from tissue incubates are outlined. Dinitrophenol, methylene blue, and brilliant cresyl blue increased the level of hepatic glycogen in rats when administered at the beginning of a 28-hour fast period. These increments are in addition to the increase caused by the cortical hormones and may represent a failure to utilize the glycogen during the fast.

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Hyperlipoproteinemia and Cholesterol Deposition at the Arteries of I^{131}-Treated Dogs.* (23644)

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Steiner, Kendall and Bevans(1) confirmed and extended their original observation, that thiouracil administration to cholesterol-fed dogs will induce atherosclerosis that is distributionally and microscopically similar to the human disease. These authors pointed out that thiouracil feeding alone, for a 14-15 month period, resulted in an average blood cholesterol concentration of 394 mg % (range 176-594) and failed to cause arterial lesions. The appearance of early atherosclerotic plaques was noted, however, after feeding cholesterol without thiouracil for a 16-month period, when an average blood cholesterol concentration of 429 mg % (range 212-736) was observed. Bevans, Davidson and Abell(2) reported a rough correlation between degree of hypercholesterolemia, length of time on experiment and extent of lesions produced in thiouracil-cholesterol-fed young dogs.

Preliminary studies in this laboratory indicated that administration of I^{131} alone would maintain an average serum cholesterol concentration exceeding 430 mg % in dogs over roughly a 12-month period. Such a preparation seemed to offer some advantages, in that the animal would remain free of the uncontrolled, generalized succinoxidase depression of thiouracil and the hepatotoxic effects of a high cholesterol diet. Even if arterial lesions were not observed after 12 months at the hypercholesterolemic level achieved, it might be possible to assess efficacy of the I^{131} treatment on the basis of altered concentrations of coronary artery and aortal cholesterol, as estimated chemically.

Methods. Each of 14 adult mongrel dogs (7 males and 7 females) was given 8 mc of

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TABLE I. Serum Concentrations of Lipid and Lipoprotein Parameters Prior to I^{131} Administration and Corresponding Average Maintenance Levels during the Subsequent Experimental Periods.

			Lipoproteins (mg %)			
			High density	Low density, P <1.063 g/cc		
	Lipid P (mg %)	Chol. (mg %)	P >1.063 g/cc	S_f 0-12	S_f 12-20	S_f 20-400
Pre-treatment						
7 ♂ (means & S.E.)	12.8 ±1.3	149 ±14.4	361 ± 45.5	41 ± 6.5	0	4 ± 2.7
7 ♀ "	15.8 ± .93	190 ± 8.3	395 ± 65.8	56 ± 9.1	0	10 ± 4.2
14 ♂ ♀ "	14.3 ± .82	169 ± 9.8	378 ± 43.8	49 ± 5.7	0	7 ± 3.3
Dog #	Exp. period (mo)	Δ wt (%)	Avg maintenance levels after I^{131}					
176 ♂	13	+93	25.0	586	503	357	260	317
96 ♂	13	+15	26.2	559	589	305	174	374
197 ♂	12	- 9	13.2	161	589	61	0	3
635 ♂	11	+38	23.1	467	680	385	182	215
526 ♂	11	+21	19.2	337	666	384	36	44
580 ♂	11	+16	18.2	324	606	291	59	46
220 ♂	11	+71	24.0	479	593	423	214	249
Avg ♂	11.4	+35	21.3	416	604	315	134	178
491 ♀	11	+41	20.6	410	595	424	44	52
599 ♀	12	+27	25.7	562	634	487	171	91
10 ♀	12	- 20	17.4	289	640	174	54	83
494 ♀	12	+38	28.0	509	731	551	147	114
633 ♀	11	+67	23.2	493	680	494	164	173
627 ♀	11	+46	22.5	412	539	489	92	54
49 ♀	11	+46	26.9	539	749	539	150	144
Avg ♀	11.3	+35	23.5	459	653	451	117	102
Avg ♂ ♀	11.4	+35	22.4	438	628	383	125	140

carrier-free I^{131} in a single oral dose and maintained on commercial dog chow and water *ad libitum* over approximately a 12-month period. The animals were weighed and initial blood samples taken prior to administration of the isotope and the weighing and blood sampling were repeated at 4-week intervals throughout the year. The blood sera were analyzed chemically for levels of cholesterol and lipid phosphorus(3) and ultracentrifugally(4) for concentration of high and low density lipoproteins. The initial serum samples were labeled "pre-treatment"; the analytical results were averaged for males, females and in combination, and the means recorded in Table I. The periodic readings for each serum lipid and lipoprotein parameter recorded for each dog throughout the experimental year were combined into an "average maintenance level" (arithmetic mean of all observations) and are so listed in Table I. At term, all dogs were sacrificed by rapid arterial exsanguination, autopsied and tissues were taken for analysis. The coronary artery was excised from the heart, carefully cleaned of adhering fat and connective tissue and divided at the coronary sinus. One branch was taken for histologic examination, and the other analyzed for cholesterol concentration. The aorta was similarly excised, cleaned and analyzed. A portion of the liver was taken for analysis of cholesterol concentration. 300-500 mg of tissue (wet weight) were dissolved in 1 cc of 50% aqueous KOH, the resulting colloidal sol washed quantitatively into 25 cc of alcohol-acetone and the cholesterol analysis completed in micro-glassware by the Sperry-Schoenheimer(5) technic. Repeated control studies in our laboratory demonstrated that when at least 80 γ of cholesterol were precipitated with digitonide all recoveries ranged between 95-105%. At regular intervals throughout the experimental year a total of 26 adult male dogs were sacrificed for control concen-

trations of aortal, coronary artery and liver cholesterol.

Results. Excepting dog #197 and possibly #10, the I^{131} animals developed gross signs of thyroid deficiency. The data in Table I demonstrate an average 30% increase in weight associated with a generalized edema and an average maintenance serum cholesterol concentration of 438 mg %. Correspondingly, the average maintenance levels of lipid phosphorus and all classes of ultracentrifugally determined lipoproteins are seen to be significantly higher after I^{131} administration. The alterations observed in the lipoprotein patterns are consistent with the reports of Lewis *et al.*(6). The only male-female difference in a serum parameter response was recorded in the significantly higher concentrations of the 0-12 class of lipoproteins achieved in the females. #10 was the only female for which a 0-12 lipoprotein concentration was observed at a level less than the highest value for the same serum parameter among the 7 male dogs.

Gross and microscopic examinations of the coronary arteries and aortae of I^{131}-treated dogs failed to demonstrate atherosclerotic lesions. There was no evidence of fatty degenerative or infiltrative changes in the liver. On the other hand, in the experimental male dogs, cholesterol analyses of these tissues revealed significant differences from those of the control males. Though the absolute differences are not striking, they are highly significant. The mean cholesterol concentration in the coronary arteries of the experimental male group was 2.0 $\pm$ 0.16 mg/g wet weight, as compared with the male control mean of 1.5 $\pm$ 0.076 mg/g wet weight ($P<0.01$). Similarly, mean aortal cholesterol concentration in this experimental group was 1.8 $\pm$ 0.074 mg/g wet weight, compared with 1.5 $\pm$ 0.033 mg/g wet weight in the control group ($P<0.01$). Since no attempt was made to separate adventitia, media, and intima and analyze these separately, it is not known if these concentration differences were uniform in the vessel wall layers, or if there was selective concentration in a portion of the vessel walls of the treated male dogs. It is possible that a selective deposition occurred in the intima, (as occurs in the natural atherosclerotic process), and that the results might have been more striking had the vessel layers been separately analyzed.

It was noted that some fatty infiltration of liver occurs after I^{131} administration that is apparently imperceptible by standard staining technics. The difference between the average of 3.2 mg/g wet weight recorded for livers of treated male dogs was significantly higher ($P<0.01$) than the average liver cholesterol level (2.3 mg/g wet weight) found for control animals.

Comparison of the blood lipid and arterial cholesterol levels demonstrates that, in this study, there is no single blood lipid parameter that can be correlated, on an individual basis, with the levels of cholesterol found in either vessel wall at autopsy. Such is not surprising in view of the fact that only the relatively recent intimal cholesterol deposition is assumed to be the tissue parameter measured. Analysis of intimal tissue alone, or the measurement of radio-cholesterol deposition in the vessel wall would probably be more sensitive indices of recent intimal deposition. Accordingly, studies along these lines are proceeding in this laboratory.

Summary. Dogs maintained for a year after I^{131} administration were found to achieve average maintenance levels of serum cholesterol in excess of 430 mg % and comparably elevated concentrations of other serum lipid and lipoprotein parameters. Tissue analysis at autopsy revealed significantly elevated levels of coronary artery, aorta, and liver cholesterol in male treated dogs, as compared with control males. Treated female dogs differed from treated males in developing significantly higher levels of S_f 0-12 blood lipoproteins, and slightly, possibly significantly, lower levels of coronary artery cholesterol. No correlation between altered tissue cholesterol content and any of the serum lipid or lipoprotein moieties was observed.

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Behavior of Explants from Human Adult Bronchial Epithelium *in vitro*.* (23645)

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During the last few years several strains of normal and carcinomatous human tissues have been maintained continuously in tissue cultures(1,2,3,4). None of these strains have been derived from non-neoplastic bronchial epithelium.

The following paper presents observations on the behavior of explants from adult human bronchial epithelium and reports the establishing of 2 permanent strains of such epithelium.

Material and methods. Samples from the bronchus of patients undergoing removal of a lung or a lobe of the lung were obtained from the operating room under aseptic conditions. The epithelium was stripped from a 1.5-2 cm length of the most proximal part of the bronchus. The small fragments, consisting of bronchial epithelium and submucosal tissue, were treated for 10-15 minutes with 5 ml of a freshly made 0.1% solution of trypsin in Hanks' salt solution containing 20 mg streptomycin and 20,000 units of penicillin. The fragments were then explanted into chicken plasma-chicken extract clot to which 40% human serum medium was added. The human serum was pooled from 2 or 3 donors, diluted with Hanks' solution containing 50 mg streptomycin and 50,000 units of penicillin per 100 ml. From the several methods of tissue-culture tried the use of micro Petri dishes(5) proved to be most satisfactory for the primary explants in our experiments. Perforated cellophane discs were put into these dishes to support the cultures since the growing cultures liquefied the clots. During 24 to 48 hours the phenol red indicator in the medium turned yellow showing acid production in the growing cultures. The medium was replaced daily or every other day by fresh 40% human serum medium. If a primary explant was established, subcultures in roller tubes were attempted after 7 to 10 days.

Results. Cultures from the bronchial epithelium of 119 adults were made. During the first 24 hours large numbers of round cells wandered to the periphery of the explant. Active movement of the cilia of ciliated epithelium could be observed. In many cultures no further migration of the cells occurred and the explant died in 4-6 days. If the explant became established, sheets of epithelial tissue grew out from the periphery of the cultures from the second day onward. The clones had blunt and rounded periphery, and the adjacent cells were without visible intercellular substance and were polygonal in shape. Many of the outgrowths ceased to increase after a few days, several survived 2 to 3 subcultures up to 40-50 days. Two permanent strains were established. Ciliation was often observed in the second or third subcultures, and both permanent strains contained ciliated epithelial cells up to the 6th generation. Such differentiation was not seen in the subsequent rapidly growing substrains. The microscopic appearance of the first outgrowth did not show any appreciable difference between the strains which ceased to grow and the 2 strains which gave rise to permanent strains.

Table I shows that apparently normal bronchial epithelium derived from persons having

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