Acta, 1954, v14, 12.

6. Harel, J., Vigier, P., *Bull. Canc.,* 1953, v40, 186.

7. Eckert, E. A., Sharp, D. G., Green, I., Beard, J. W., *J. Nat. Canc. Inst.,* 1955, v16, 593.

8. Engelbreth-Holm, J., Oliver and Boyd, Ltd., Edinburgh, *Spontaneous and Experimental Leukaemia in Animals,* 1942.

9. Green, I., Beard, D., Eckert, E. A., Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 406.

10. Benedetti, L. E., Bernhard, W., Oberling, Ch., *C. R. Acad. Sci.,* 1956, v242, 2891.

11. Bernhard, W., Oberling, Ch., Vigier, Ph., *Bull. Canc.,* 1956, v43, 407.

12. Gaylord, W. H., *Canc. Res.,* 1955, v15, 80.

13. Rouiller, Ch., Haguenau, Fr., Golde, A., Lacour, F., *Bull. du Canc.,* 1956, v43, 10.

14. Oberling, Ch., Bernhard, W., Vigier, Ph., *Nature,* v180, 386.

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Influence of *in vivo* Administration of Adrenocorticoids on Metabolism of Thymus, Liver and Tumor Tissues.* (23643)

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Prominent among approaches employed in investigations of the metabolic functions of adrenocortical hormones have been measurement of various metabolic products following administration of the hormones to live animals, and measurement of activity of specific enzymes or enzyme groups after hormone administration *in vivo* or *in vitro*(1,2). In the present studies, the hormones were administered *in vivo,* usually after adrenalectomy, and tissues were removed 4-6 hours later for *in vitro* metabolic studies. Tissues of adrenalectomized animals were employed for obtaining low-hormone control data. Because of the lability of lymphoid tissue to the action of adrenal corticoids, thymus tissue was given particular attention in anticipation of high metabolic sensitivity to cortisone preceding morphological changes(3).

Methods. Cortisone acetate (cortisone) or cortisone in combination with hydrocortisone was administered to 125-175 g male rats of the Holtzman strain or young adult male mice from the Stokely-Peterson colony 4 to 6 hours before the animals were killed and the tissues removed. The hormones, 10 mg per rat and 1 mg per mouse, were injected half by the intraperitoneal and half by the subcutaneous routes(4). When adrenalectomized animals were employed the operations were performed 3 to 5 days previous to hormone administration to allow for recovery from the operation with negligible increase in accessory corticoid-secreting tissue. After adrenalectomy the animals received 1% NaCl and 5% glucose in the drinking water for 24 hours and 1% saline thereafter. Purina laboratory chow was fed *ad libitum.* No adrenalectomies were performed in tumor-bearing animals. Cell suspensions of thymus, liver, or Murphy-Sturm lymphosarcomas from rats, prepared by the procedure of Kit and Barron(3), and Ehrlich ascites cells, harvested from mice, were washed and pipetted into Warburg vessels containing the radioactive substrate in a side arm. The flasks were covered with serum caps through which a 95% O_2-5% CO_2 mixture was introduced by hypodermic needle. All preparative manipulations were performed in the cold, and incubations were carried out in Krebs-Ringer bicarbonate buffer(5) for 60 minutes at 37.5° with shaking. At the end of the incubation 0.2 ml of 10% KOH was added to the center well and 0.3 ml of 70% perchloric acid (PCA) to the incubation medium by hypodermic syringe and long needle. The side arm was rinsed with the acidified mixture, and the flasks were shaken for an additional hour to insure the complete libera-

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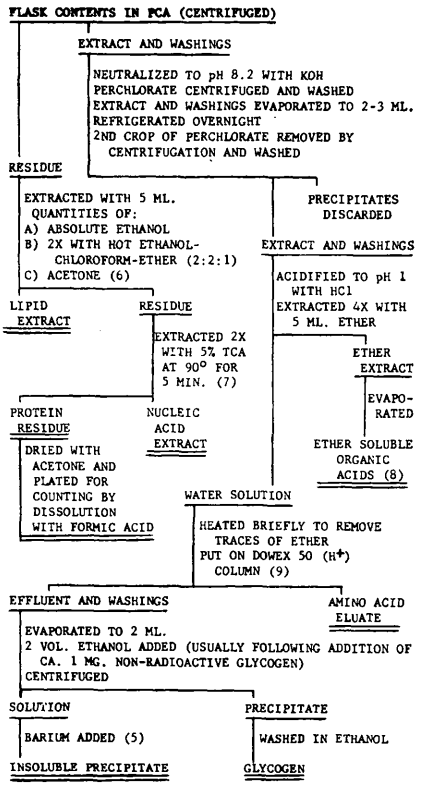

FIG. 1. Isolation of metabolic components from incubation mixtures for radioactivity assays.

tion of CO_2. The contents of the center well were removed and assayed for $C^{14}O_2$. The mixture from the main compartment was transferred to a centrifuge tube and carried through the procedures described in Fig. 1. Two dimensional descending paper chromatograms (Whatman No. 1 paper) were run employing ethyl acetate-acetic acid-water (4:4:1) *vs.* butyric acid-sodium butyrate-water (10) for the barium-ethanol insoluble residues and 80% phenol *vs.* butanol-acetic acid-water (8:2:5) for the amino acids. For the ether soluble fraction one-dimensional chromatograms were employed with a phenol-formic acid-water (79:2:19) system. Radioactive spots were located on the chromatograms by exposure of x-ray films(11), and in several instances the spots thus located were cut out, eluted and counted quantitatively. Liver glycogen was determined as glucose after treatment of the tissue with hot alkali. The counting of radioactive samples and chemical determination of glucose have been described(4).

Results. The first experiment was designed to determine the effect of the experimental procedures on the morphology of the thymus. Male mice 9 weeks of age were given a single dose of 1 mg of cortisone as described above. The weight of thymus glands of groups of mice was determined periodically thereafter. As shown in Fig. 2, a rapid weight loss began between 6 and 9 hours and at 24 hours the gland was reduced to a third its original weight. Microscopic examination of these same glands revealed insignificant disintegration of lymphocytic cells before 24 hours. A similar study has been reported(12).

From the metabolites examined, consistent differences in the metabolism of the tissues of the adrenalectomized and adrenalectomized, hormone-treated animals were demonstrated with respect to carbon dioxide production, glycogen formation, and incorporation of radioactivity into protein. With glycine-2-C^{14} or uniformly labeled glucose as the source of radiocarbon, decreased accumulation of radioactivity into protein and carbon dioxide by thymus tissue resulted from treatment with the adrenal hormones (Table I). Similar decreases in incorporation of radioactivity into these 2 pools under the influence of hormone treatment were observed with acetate-1-C^{14}

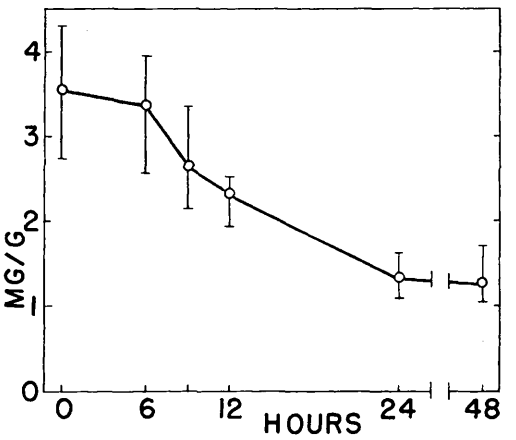

FIG. 2. Effect of a single dose of cortisone (1 mg) on wt of thymus glands. Each exp. group consisted of 4 mice while 6 mice served as controls; 3 control mice were killed at 6 hr and 3 at 24 hr. The wt of the glands are expressed as mg/g body wt, with both range and avg shown.

TABLE I. Some *In Vitro* Effects of Cortisone Administered *In Vivo*.

Tissue, substrate and metabolic pool	Adrenalectomized (cpm/mg dry tissue)*	Adrenalectomized + cortisone (cpm/mg dry tissue)*
Thymus, glycine (.0013)†‡		
CO_2 (97 ± 4)§	98 ± 0	76 ± 1
Protein (3160 ± 115)	4105 ± 259	1962 ± 175
Lipid (85 ± 2)	92 ± 5	64 ± 5
Thymus, glucose (.0037)		
CO_2	2425 ± 180	1493 ± 221
Protein	359 ± 26	180 ± 35
Lipids	94 ± 6	55 ± 7
Nucleic acids	74 ± 1	52 ± 4
Ether soluble	1265 ± 96	1563 ± 286
Free amino acids	2662 ± 451	2867 ± 222
Glycogen	41 ± 17	130 ± 18
Ba^{++}-Ethanol Ppt.	13900 ±4270	14750 ±3200
Liver, fructose (.0037)		
CO_2	139 ± 37	83 ± 38
Protein	529 ± 17	488 ± 80
Ether soluble	1395 ± 126	1520 ± 224
Free amino acids	242 ± 37	356 ± 87
Glycogen	30 ± 3	45 ± 1
Ba^{++}-Ethanol Ppt.	5330 ±1110	8350 ±2650
Murphy-Sturm, acetate (.00092)‡		
CO_2	4975 ± 65	2095 ± 660
Protein	617 ± 50	360 ± 156
Nucleic acids	67 ± 18	23 ± 9
Murphy-Sturm, glycine (.0013)‡		
CO_2	14 ± 2	8 ± 1
Protein	476 ± 153	114 ± 30
Lipids	65 ± 16	86 ± 4
Nucleic acids	57 ± 28	23 ± 11
Ehrlich ascites, acetate (.0013)‡		
CO_2	603 ± 12	725 ± 27
Protein	98 ± 4	139 ± 5
Lipids	316 ± 110	413 ± 36
Ehrlich ascites, glycine (.0013)‡		
CO_2	76 ± 4	113 ± 7
Protein	3495 ± 195	3605 ± 206
Lipids	496 ± 40	484 ± 13

* Values were obtained from duplicate and triplicate determinations on tissue pools made from as many as 8 (usually 4-6) animals. All values are corrected to 3.0×10^6 c.p.m./flask and are averaged ± stand. error of mean. Although stand. errors are included in the data, the experiments were designed to survey broad areas rather than to establish precise values.

† Radioactive substrate at molarity indicated. In addition flasks contained bicarbonate buffer, 35-65 mg dry wt of tissue, and glucose if indicated.

‡ Non-radioactive glucose added to incubation mixture at approximately physiological level, 0.0056 M.

§ Values for intact untreated controls.

‖ Cortisone treatment 24 hr before sacrifice (instead of usual 4-6 hr).

or uniformly labeled fructose as substrate (not illustrated). While liver tissue also showed decreased incorporation of radioactivity into the CO_2 and protein pools in some experiments, the pattern was not consistent under the experimental conditions employed. Liver tissue, however, regularly accumulated increased radioactivity into its glycogen component under the influence of the hormone. Although predictable differences were not observed for any of the other metabolites investigated, the hormone administration was often accompanied by decreased synthetic activity in thymus tissue particularly as demonstrated by the lipid fraction.

The tissue preparations derived from Murphy-Sturm lymphosarcomas (Table I) produced radioactive CO_2 and incorporated radioactivity into protein at uniformly lower rates as a result of hormone administration. In an experiment not shown in the table, Gardner lymphosarcoma ascites cells harvested from C3H mice also exhibited an inhibition in rate of metabolism resulting from cortisone treatment. Erlich ascites cells, not derived from lymphatic tissue, were no more consistent than liver in the effects of the hormone treatment.

Autoradiograms made from the free amino acid fractions, the ether soluble fractions, and the barium-ethanol precipitates showed no qualitative or significant quantitative differences in the individual metabolites produced by the tissues from animals with or without hormone treatment.

Since the influence of the adrenal hormones upon synthesis of glycogen by liver tissue was demonstrable even during *in vitro* incubations, it was of interest to continue the studies with special attention to this constituent. Dianzani and Scuro(13) reported that 2,4-dinitrophenol (DNP) administered for 6 days to rats increased the storage of liver glycogen. Several other compounds studied by these workers were also of interest as inhibitors of various phosphate transfer reactions, most of which have been implicated in some way with the action of the adrenal hormones.

When DNP, brilliant cresyl blue (BCB), and methylene blue (MB) were administered intraperitoneally to fasting, adrenalectomized

TABLE II. Influence of Metabolic Inhibitors on Glycogen Production in Livers of Adrenalectomized Rats.

Treatment*	Liver glycogen (mg/g fresh tissue)
Adx	.7 ± .1
" + hormones	6.5 ± 1.2
" + DNP	4.1 ± .2
" + DNP + hormones	16.0 ± 2.6
" + MB	1.9 ± .4
" + MB + hormones	10.7 ± 1.6
" + BCB	1.3 ± .4
" + BCB + hormones	16.4 ± .2

* Typical experiment: 4 animals/group; animals fasted 28 hr and hormones admin. 4 hr before sacrifice; 5 mg cortisone and 5 mg hydrocortisone/rat.

rats (Table II) it was observed that DNP, given in 2-4 mg doses at the beginning of a 28-hour fast, increased the liver glycogen to 6 to 10 times the level in untreated adrenalectomized animals. MB or BCB at equimolar levels had significant but lesser effects (80-150% increases). None of the 3 inhibitors influenced the increase in glycogen content of the livers by cortisone or hydrocortisone administered 4 hours before sacrifice. Since the inhibitors are very toxic to the rats when administered during the latter part of the fast period, no evidence could be obtained to indicate that these substances increase the glycogen level in the manner of the adrenal hormones after the level has been reduced by the fast; rather they apparently prevent part of the decrease during the fasting period. Because of its toxicity Janus green B could not be studied satisfactorily at the levels employed.

Discussion. Results of the present study indicate that adrenalectomy and adrenalectomy plus adrenal cortical hormone treatment altered the metabolism of certain animal cells during a subsequent incubation *in vitro*. The metabolic changes occurred prior to the onset of rapid destruction of lymphocytes. Therefore these changes reflect a true metabolic response to cortisone rather than the result of altered tissue structure due to prolonged cortisone action.

The depression in protein synthesis (or build-up of radioactivity into protein) by the adrenal hormones in thymus tissue or tumors of lymphatic origin may be a manifestation of the phenomenon observed by Roberts(14) in which cortical extract or adrenocorticotropin enhanced the release to the medium of protein by incubating tissues. Kit and Barron(3), who also utilized metabolizing cell suspensions but added the cortical hormones *in vitro*, reported that respiration of lymphatic cells and incorporation of C^{14} into protein from acetate, glycine, or phenylalanine was inhibited, but incorporation of C^{14} into lymphosarcoma cells was depressed only by higher hormone levels. In the present study, in which the amounts of hormones administered to the whole animals made it unlikely that quantities of the hormones comparable to those employed by Kit and Barron reached the tissues taken for incubation, similar or larger inhibitory effects on $C^{14}O_2$ production and incorporation of radioactivity into protein resulted.

The accumulation of glycogen observed in the liver may result from depression of processes accounting for the utilization or turnover of hepatic glycogen. Such a possibility is substantiated by the series of compounds such as DNP which, in spite of their inhibitory effects, increased the glycogen level in livers of fasting rats. The incidental observation of the consistently high metabolic rate of thymus tissue compared to liver marks this tissue as one deserving more study. Also to be noted is the difference in influence of adrenal hormones on the various normal tissues of the body. This variability in response indicates that a variety of tissues should be studied in investigating the basic action of the hormones.

Summary. In cell suspensions of thymus or lymphatic tumor tissues from rats treated 4 to 24 hours previously with cortisone or hydrocortisone consistent decreases in $C^{14}O_2$ production and in incorporation of C^{14} into tissue protein were demonstrated whether incubations were conducted in the presence of uniformly labeled glucose or fructose, acetate-1-C^{14} or glycine-2-C^{14}. Liver tissue exhibited increased *in vitro* accumulation of C^{14} into glycogen after hormone treatment. A variety of other metabolic pools showed no difference in total C^{14} accumulation or distribution re-

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.

1. Sayers, G., *Physiol. Revs.*, 1950, v30, 241.
2. Swingle, W. W., Kleinberg, W., *Ann. Revs. Physiol.*, 1955, v17, 367.
3. Kit, S., Barron, E. S. G., *Endocrinol.*, 1953, v52, 1.
4. Boutwell, R. K., Chiang, R., *Arch. Biochem. and Biophys.*, 1954, v50, 461.
5. Umbreit, W. W., Burris, R. H., Stauffer, J. F., *Manometric Techniques and Tissue Metabolism*, Burgess Publishing Co., Minneapolis, 1949.
6. Zamecnik, P. C., Loftfield, R. B., Stephenson, M. L., and Steele, J. M., *Cancer Res.*, 1951, v11, 592.
7. Schneider, W. C., *J. Biol. Chem.*, 1945, v161, 293.
8. Katz, J., Chaikoff, I. L., *ibid.*, 1954, v206, 887.
9. Kit, S., *Cancer Res.*, 1954, v14, 397.
10. Wade, H. E., Morgan, D. M., *Biochem. J.*, 1955, v60, 264.
11. Benson, A. A., Bassham, J. A., Calvin, M., Goodale, T. C., Haas, V. A., Stepka, W., *J. Am. Chem. Soc.*, 1950, v72, 1710.
12. Ringertz, N., Fagracus, A., Berglund, K., *Acta Path. Microbiol. Scand.*, 1952, Supp. 93, 44.
13. Dianzani, M. U., Scuro, S., *Biochem. J.*, 1956, v62, 205.
14. Roberts, S., *J. Biol. Chem.*, 1953, v200, 77.

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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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