A third group of animals, similarly treated, was sacrificed at the end of 29 days, and the kidneys, adrenals, and other organs and tissues removed for weighing and histological examination. The average weight of the kidneys in the group run daily at 15,000 feet was 945 ± 14 mg (probable error of the average) per 100 g body weight; while the average weight of the kidneys in a series of normal animals, under precisely similar dietary and other conditions, save that these control rats were kept continually at room pressure, was 870 ± 14 mg. The kidneys of the experimental animals were 75 mg heavier than those of the controls, and this difference is statistically significant (probable error of the difference, ± 20).

The polyuric response of white rats to daily 3-hr exposure to an altitude of 15,000 feet is thus seen to be accompanied by renal hypertrophy. It should be emphasized that the conditions of the experiment were not critical. The animals emerged from each daily sojourn in the low-pressure chamber with no symptoms of strain or malaise, and during the course of the protracted experiment they gained weight normally and remained in excellent health. Nevertheless, these conditions (daily 3-hr exposure at 15,000 feet) were apparently severe enough to lead not only to hypertrophy of the kidneys, but also to hypertrophy of the adrenal glands and lymphoid tissue, findings which will be reported later by Dr. J. E. Kindred of the Department of Anatomy in another connection.

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The Search for a Physiologically Active Substance in Calf Thymus Tissue.

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During 1940, Bomskov and associates published a series of articles[1] in which they described a new hormone obtained by extracting fresh calf thymus tissue with lipoid solvents. Complete details of the method of preparation were not given. The hormone mentioned is described as the mediator of the pituitary diabetogenic effect, the effector of blood sugar elevation, and the initiator of intense growth. Involution of male gonads and adnexa and inhibition of the effect of the thyrotropic hormone follow its use. Sensitivity to chloroform is increased. The hormone is said to be transported by an increased number of circulating lymphocytes and to be detectable in small quantities of urine. In the tadpole growth is stimulated but metamorphosis is retarded. Greatest secretion and consequent urinary excretion is said to occur in those clinical states which exhibit most marked lymphocytosis, in carcinoma, and in Hodgkin's Disease.

Very recently Wells and associates[2] reported their inability to confirm Bomskov's results. Using the rat and Carneau pigeon as experimental animals they failed to observe any significant blood sugar or liver glycogen variations, ketonemia, or any constant leucocytosis following injection of their extracts. Neither tissue equivalents of extracts used nor nonspecific control tissue extracts were mentioned by these investigators.

The present study was begun two years ago in an attempt to establish the presence of a physiologically active, lipoid solvent soluble

[1] *a.* Bomskov, C., and Sladovic, L., *Deut. Med. Wchnschr.*, 1940, **66**, 589; *b.* Bomskov, C., and Holscher, B., *Z. Klin. Med.*, 1940, **137**, 745; *c.* Bomskov, C., and Sladovic, L., *Pflüger's Arch.*, 1940, **243**, 611; *d.* Bomskov, C., and Brachat, F., *Endok.*, 1940, **23**, 145; *e.* Bomskov, C., and Karl-Heinz, K., *Pflüger's Arch.*, 1940, **243**, 623; *f.* Bomskov, C., *Pflüger's Arch.*, 1940, **244**, 246; *g.* Bomskov, C., *Endok.*, 1941, **23**, 239; *h.* Bomskov, C., *Endok.*, 1941, **23**, 225.

[2] Wells, B. B., Riddle, O., Marvin, H. N., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 473.

substance in thymus tissue. Several months after this work was initiated the first paper by Bomskov *et al.*[1] came to our notice and it was apparent that our investigations were to a great extent parallel in purpose and method.

Preparation of the Extract. Approximately 125 lbs. (57 kg) of fresh frozen calf thymus tissue was finely ground in 10-lb. lots and extracted using one liter of 95% ethyl alcohol or one liter of acetone or one liter of equal parts of alcohol and acetone per lb. of tissue. Seventy-five pounds of kidney and lung tissue were similarly extracted for use as control tissue extracts. As a further control we used appropriate quantities of the vehicle, corn oil, in which the extracted residues were dissolved. The initial extraction mixtures of tissue and alcohol were agitated for 6 hr and then placed in a cold chamber at 3° C for 18 hr. The solvent was then pressed off in a small tissue press and the alcohol was immediately taken off under vacuum at 40-50° C at a pressure of 15 mm of mercury. A preliminary experiment in which 25 lb. of thymus tissue was extracted as described above with acetone was undertaken. Upon removal of the acetone, the residue was extracted with ethyl ether and the ether soluble residue was taken up in corn oil. The aqueous residue of the remaining 100 lb. was extracted 6 to 8 times with one-half volumes of petroleum ether. The petroleum ether was removed and the residue saved for injection and for further fractionation into 70% alcohol soluble, petroleum ether soluble, and benzene soluble fractions. The aqueous residue following removal of petroleum ether soluble substances was then extracted with 1, 1, trichlorethane, boiling point 113°. The 1, 1, trichlorethane was then removed under vacuum and the residue saved for injection and further fractionation into petroleum ether and 10% alcohol soluble fractions. Ethyl ether and benzene extractions of the remaining aqueous residues were made in 3 ten-lb. lots following the procedures already described above.

Methods. Possible evidence of metabolic changes following and attributable to subcutaneous injections of thymus extracts was sought through these several experimental approaches: (1) Blood sugar determinations in the rat at 1, 3, 9 and 24 hr after injection; (2) insulin sensitivity in the mouse[3] and (3) rat, as titrated by injections of 20 U of insulin per kg per hr with serial blood sugar determinations until convulsions occurred; (4) the blood sugar reaction of the diabetic rat (animals made diabetic by partial pancreatectomy, by 14 daily injections each of 30 U of insulin per kg, and by starvation for 7 days); (5) changes in leucocyte equilibria; and (6) the relative weights and histology of the endocrine organs, liver, spleen, and kidneys following injections of extract.

Results. The effect of the thymus extracts on the blood sugar of 58 rats fed and fasted was tested. Nine experimental rats received 100 g tissue equivalents of ethyl ether soluble substances in corn oil obtained directly from aqueous residues following the removal of acetone. The 9 control animals received only corn oil. Blood sugar values in fed and fasted animals at 1, 3, 6, 9, and 24 hr after injection showed no significant variations from those observed in the controls. Twenty experimental and 20 control animals were given thymus 1, 1, trichlorethane soluble substances in tissue equivalents of 150-200 g, petroleum ether soluble substances in tissue equivalents of 150-200 g, and aqueous residues in tissue equivalents of 30-60 g. The fat soluble substances produced no significant changes in blood sugar taken 1, 3, 6 and 9 hr after injections, as compared with controls treated with similarly prepared tissue control extracts. Intraperitoneal injection of thymus aqueous residues produced blood sugars above 250 mg but similar levels were obtained with control tissue aqueous residues.

The insulin sensitivity of 120 mice[3] (60 experimental and 60 control) was tested using 100-150 g tissue equivalents of 1, 1, trichlorethane soluble substances. Other fractions of the extract were tried but found to be too toxic for use in this experiment. Thymus extracts gave a 25% protection in terms of survival time as compared with

[3] Jensen, H., and Grattan, J. F., *Am. J. Physiol.*, 1940, **128**, 270.

corn oil, but similarly prepared kidney extracts in the same tissue equivalence gave 75% protection as compared with corn oil. Ethyl ether soluble substances extracted from thymus aqueous residue after the removal of petroleum ether and 1, 1, trichlorethane soluble substances also showed some protection which was, however, equal to or less than that of the control tissues.

Rat insulin sensitivity was tested using 40 rats (20 experimental and 20 control) injected with all of the fractions noted above. None of these gave even suggestive protection. Blood sugar samples were taken hourly until the onset of convulsions.

The effect of the extracts was analyzed in a study of 15 diabetic animals. Three were made diabetic by partial pancreatectomy, 6 by injection of 30 U per kg of regular insulin daily for 2 weeks, and 6 by starvation for 7 days. Petroleum ether soluble substances were tried in the depancreatized animals and the results were negative. Trichlorethane soluble substances were tested in the remaining temporarily diabetic animals and again no significant elevation in the blood sugar was noted as compared with the controls. Blood sugar samples were taken at 1, 3, 6 and 9 hr, following injection of 200 g tissue equivalent of extract.

In studying the weights of endocrine glands, spleen, liver, and kidneys, 54 animals (27 experimental and 27 control) were injected daily for 18-21 days with 125-75 g tissue equivalents of the extracts described. Thymus weights in animals receiving the petroleum ether soluble fraction were found to be about 50% below and adrenal weights 50% above those injected with corn oil alone. No significant changes in endocrine size were noted as compared with control animals and experimental and control liver and spleen weights were similarly proportionate to total experimental and control body weights. Thymus size was readily correlated with toxicity in all extracts used, both experimental and control.

Changes in body weights were recorded in all animals injected over long periods and in no instance was any experimental group heavier than its control.

White blood counts and differential studies were made in a sampling from each group of animals and no significant differences were noted.

Discussion. Certain of the effects attributed to "thymhormon" by Bomskov may be nonspecific. For example, it has been found that destructive irradiation of the thymus produces profound cytologic and functional changes in the near-by thyroid gland[4] and to a lesser extent in the hypophysis. The resulting endocrine changes may be responsible for the claim that the thymus gland is the mediator of the pituitary diabetogenic effect. Furthermore, it should be noted that injection of any substance of sufficient toxicity may cause retrogression of secondary sex characteristics.

Recent studies by Rawson *et al.*[5] suggest that whole thymus tissue will inactivate the pituitary thyrotropic hormone, an effect not observed with tissues other than the thyroid gland itself. This may or may not bear a relation to Bomskov's observation that "thymhormon" inhibits the effect of the thyrotropic hormone.

In considering thymus tissue extracts as a whole, it is felt that the lipoid extracts described in the literature previously were too toxic to serve any physiologic function. This conclusion is suggested by the resulting thymus atrophy, adrenal hypertrophy, weight loss, and tissue reaction at the site of injection following their use. We chose an acetone and (or) alcohol, petroleum ether, trichlorethane method for extracting a great part of our tissue since it has been previously shown to be satisfactory in the making of potent and non-toxic extracts from adrenal cortex tissue.[6]

Summary. None of the metabolic functions studied have been significantly or specifically influenced by extracts of the thymus gland under the conditions cited.

[4] Hashimoto, E. I., and Freudenberger, C. B., *J. A. M. A.*, 1939, **112**, 1680; Jolles, B., *Am. J. Roentgen.*, 1941, **45**, 259.

[5] Rawson, R. W., Sterne, G. D., and Aub, J. C., *Endoc.*, 1942, **30**, 240.

[6] Thatcher, J. S., Dept. of Physiol., Ohio State University, personal communication.