plated out at the end of the experiment, as a routine procedure, retaining only those showing negative results.

We find by this method of measuring tissue metabolism that the blood from an alligator injected with thyroxin (Squibbs) 3 days or so previously to the test may show an increase of from 150 to 190% above the control. Direct addition of thyroxin to the blood, on the other hand, has produced no change which has an interpretable significance.

We have been unable to demonstrate that incubation of thyroxin in the blood *in vitro* has an activating effect.

Further investigation is contemplated to confirm this metabolic increase in the tissues of the injected animal and to determine the mechanism by which it is brought about.

The considerable variations among the controls are apparently due to the different seasons, different laboratory conditions, different animals used, successive use of the same animal.[15]

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Effect of Adrenal Cortical Hormone Upon Respiratory Metabolism of Adrenalectomized Cats.*

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The material here presented represents an attempt to determine the effect of bilateral adrenalectomy on the respiratory exchange of cats, together with the changes in metabolism produced by the administration of the cortical hormone to these adrenalectomized animals. There is, in the literature, some confusion as to the effect of bilateral adrenalectomy on the metabolism of animals. Golyakowski[1] reported an increase in heat production after ligation of the blood supply of the adrenals in dogs. Marine and Baumann[2] reported an increase in metabolism in 53% of their rabbits where the adrenals were removed or injured by freezing. They noted no

[15] Barron and Harrop, *J. Exp. Med.*, 1928, **48**, 207.

* This investigation has been aided by grants from the Josiah Macy, Jr., Foundation and Mrs. R. E. Breed.

[1] Golyakowski, *Vrach.*, St. Petersburg, 1899, **20**, 1017.

[2] Marine, D., and Baumann, E. J., *Am. J. Physiol.*, 1921, **57**, 135.

change in 33% and a fall in 14%. Scott,[3] using cats, obtained a slight increase in some animals and no change in others after subtotal injury of the adrenal glands, and a fall in metabolism following total removal. Aub,[4] also using cats, found a drop of 25% in the metabolism, following total removal of the adrenal glands. He did, however, detect a slight increase immediately following operation. Intravenous injection of the adrenal cortical extracts available at that time (1922) produced no effect on the metabolism of these animals.

Following the introduction of an active extract of the adrenal cortex by Swingle and Pfiffner[5] it was considered advisable to determine its effect on the metabolism of adrenalectomized cats. The operation was performed in 2 stages in the following experiments. Healthy adult animals were used. They were maintained on a constant diet and were fed approximately 20 hours before the beginning of the metabolism determination. The respiratory exchange was measured by Marine's modification of Haldane's open circuit apparatus[6] for two-hour periods. Injections of the cortical hormone were made at least 15 hours prior to the beginning of the determination of metabolism. The method of preparation of the cortical hormone used was that described in detail by Swingle and Pfiffner.[7] The adrenalin content of this extract was between one part in 1,500,000 and one part in 2,000,000. The injections were made subcutaneously.

Previous observers[2] have shown that the removal of one adrenal does not alter the level of metabolism. Following the removal of the second adrenal gland, there was no appreciable change in metabolism for from 4 to 6 days in our series of animals. From the sixth to the tenth day there was a progressive fall until a level approximately 50% below normal was reached when the animal was in a prostrate condition. Following the administration of cortical hormone to these prostrate animals, an increase in metabolism occurred within 24 to 48 hours. In from 48 to 72 hours after the beginning of the administration of the hormone, the metabolism had risen, in each case, to a level varying between 10 and 18% above the normal previously established for that individual animal. At this point the administration of hormone was stopped and there

[3] Scott, W. J. M., *J. Exp. Med.*, 1922, **36**, 199.

[4] Aub, J. C., Forman, J., and Bright, E. M., *Am. J. Physiol.*, 1921, **55**, 293.

[5] Swingle, W. W., and Pfiffner, J. J., *Anat. Rec.*, 1929, **44**, 225; *Am. J. Physiol.*, 1931, **96**, 153.

[6] Marine, D., *J. Metabolic Research*, 1922, **2**, 29.

[7] Pfiffner, J. J., and Swingle, W. W., *Am. J. Physiol.*, 1931, **96**, 180.

was a fall within 24 to 36 hours to the normal level. This was maintained for a varying time period (several days), during which the animal appeared normal. At the end of that time the metabolism began gradually to decrease, signs of adrenal insufficiency developed, and the cycle was repeated.

The changes have been studied in a series of 6 cats. One was observed through 3 cycles, one through 2 and the remainder through one. The metabolic changes were essentially the same in each case.

Further observations are in progress as to the effect of the cortical hormone on normal animals, and an attempt is being made to determine the rôle of the thyroid gland in the experiments described above.

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Solubility of Human Gall Stones in Dog Bile.

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That human gall stones dissolve in gall bladder bile of the dog has aroused considerable interest over a period of years. The reason for this phenomenon has never been accurately determined. Recently Walsh and Ivy[1] showed that the unsaponifiable fraction of dog bile which includes cholesterol, is smaller than that of human bile, while the saponifiable matter, which contains bile salts and soaps, is considerably larger. No quantitative estimation of the individual bile constituents was made.

The concentration of cholesterol in gall bladder bile of the human, beef and dog was determined by the gravimetric method of Windaus, *before* and *after* saturation with finely powdered cholesterol. This sterol is easily the most important constituent of human gall stones from the quantitative aspect. The figures in Table I indicate that while dog and ox bile contain little cholesterol they are capable of dissolving considerable quantities of this substance; this, we believe, is the chief cause for the solubility of human gall stones in gall bladder bile of the dog.

[1] Walsh, E. L., and Ivy, A. C., *Ann. Int. Med.*, 1930, **4**, 134.