When challenged again with BAS-phenol (same dose) the rise in pressure was insignificant (8 mm of Hg). Tryptamine (1.1 mg/kg), however, elicited a full response (80 mm of Hg). This experiment was repeated in another dog with similar results.

*Specificity.* In the intravenous test with dogs as described above BAS-phenol was a remarkably specific antagonist of serotonin. It did not antagonize the action of adrenaline, noradrenaline, or even of tryptamine. Data illustrating these points are being published elsewhere(10).

*Toxicity of BAS-phenol.* Acute and chronic toxicities for adult mice (10 for each dose level) given BAS-phenol intraperitoneally were determined by the methods previously described for other members of this series(9, 11). All animals were killed by a single injection of 80 mg/kg and all survived a single injection of 40 mg/kg. A daily dose of 40 mg/kg for one week killed 2 of 10. Twenty mg/kg per day for one month was well tolerated. No animals died and none lost weight or showed changes which could be detected by visual inspection of the organs on autopsy.

*Summary.* A method was described for synthesis of 1-benzyl-2-methyl-5-hydroxytryptamine involving cleavage of the methyl ether of 1-benzyl - 2-methyl - 5-methoxytryptamine (BAS) by heating with pyridine hydrochloride. The phenol so produced exhibited powerful antiserotonin action either orally or intravenously in dogs. In the intravenous test it was the most potent antiserotonin thus far encountered. It also had considerable serotonin-like activity.

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## Effect of a Brain Extract on Turnover Rate of Serum Cholesterol.*† (23503)

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At high levels of serum cholesterol a definite reduction will usually be achieved in human subjects with the feeding of an alcoholic extract of defatted brain(1). This closely simulates the effect of oral beta-sitosterol(2). However, when low normal levels of serum cholesterol already exist, a significant reduction of the total serum cholesterol may not be achieved with similar dosage. Thus the question arises whether any effect on cholesterol metabolism occurs in such cases, and further whether any effect exists in the absence of dietary cholesterol which had been used in our earlier chicken experiments(3). The following experiments were performed in an effort to elucidate the effect of this material upon the endogenous cholesterol metabolism in the experimental animal.

*Methods. Chick experiment.* An 8-week-old cockerel, on a diet of 100 g per day of starter mash, received an intravenous injec-

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tion of 51 mg of 1-$C^{14}$ sodium acetate containing 1 mc/meq. Blood samples were drawn at least twice weekly in the same postprandial state for 8 weeks. From the 21st day to the 34th day 10% of the mash was supplanted by the cerebroside extract§ previously described(1). At each bleeding at least 6 ml of blood was drawn and oxalated, the plasma was removed and duplicate 1.5 ml aliquots were precipitated and refluxed for 20 minutes in 30 ml of 3:1 alcohol:ether. The filtrate and washings were pooled, evaporated and the residue saponified under nitrogen in 10% alcoholic KOH. The unsaponifiable matter was then extracted 3 times with petroleum ether, the extract evaporated under $N_2$ and transferred to a small centrifuge tube with a few ml of ether. The ether was then allowed to evaporate under $N_2$, and the residue dissolved in 0.5 ml of absolute ethanol. The supernatant was then treated with digitonin by a modification of the method of Hellman *et al.* (4). The digitonide was then transferred as an ether slurry to preweighed planchets 3.47 cm in area and allowed to dry for several hours at room temperature and for 15 minutes at 110°C. After determining the constant weight of the digitonide, the samples were counted in a windowless gas flow counter, the counts per minute being corrected to infinite thickness of the digitonide from an experimentally determined curve.

*Dog experiment.* A 16 kg mongrel dog was injected intravenously with 42 mg of I-$C^{14}$ sodium acetate containing 1 mc/meq and, 14 days later, 0.8 mc of $Na_2HP^{32}O_4$ was injected intravenously. The constant daily diet of 207 g Purina Lab. chow plus 228 g of a commercial dog food was fed throughout, except that 40 g per day were replaced by the brain extract from the 20th to the 29th day of the experiment. Oxalated blood samples were obtained at 2- to 5-day intervals and the plasma obtained by centrifugation. Duplicate 5 ml samples were precipitated in 100 ml of a 1:1 alcohol:acetone solution which was brought to the boiling point on a water bath and filtered. The filtrate was taken to dryness and the residue extracted 4 times with small volumes of boiling petroleum ether. This extract was again taken to dryness, saponified, and the digitonide precipitate obtained and again washed as described by Hellman, Rosenfeld and Gallagher(4), except that it proved necessary to wash the digitonide twice with ethanol at 0°C to avoid contamination of the digitonide by $P^{32}$. In addition, duplicate samples of 1.5 ml of plasma were precipitated in 10 ml of trichloracetic acid for determination of the radioactive phosphorus incorporated in the phospholipid. The supernatant was discarded and the precipitate washed 3 times with 5% trichloracetic acid before being extracted 4 times with 3 ml of boiling ethanol after the method of Schneider(5). The phospholipid was then isolated by the technic of Kennedy (6). After the final washing, 4 ml of the resulting 2:1 ethanol:$CCl_4$ extract was placed in a centrifuge tube and taken to dryness. The residue was then digested in an aluminum block with 0.4 ml of 10N $H_2SO_4$ until charred, and treated with $H_2O_2$ until clear. This was then brought to a 5.5 or 10 ml volume, and an aliquot of 0.5 ml plated on a planchet for immediate counting while wet. A 5 ml aliquot was then used for determination of phosphorus by the method of Gomori(7). The planchets were counted in the gas flow counter and the counts per minute per microgram of phosphorus determined. Samples of feces were collected on the 11th and 23rd day. These were homogenized in a Waring blendor with an equal volume of hot acetone, centrifuged and the supernatant collected. The residue was further extracted with hot acetone 3 times, and then taken to dryness at room temperature, ground in a mortar and pestle and placed in a desiccator until constant weight was obtained. This weight is referred to as the "dry weight" of the feces, though it is recognized that in addition to being dry it is relatively free of easily soluble lipid. An aliquot of the acetone extract was then saponified in 10% alcoholic KOH for 20 minutes and the unsaponifiable matter extracted with 3 washings of 150 ml petroleum ether. An appropriate aliquot of the petroleum ether extract was then dried down in a preweighed centrifuge cone, redissolved in absolute alcohol and pre-

§ We are grateful to Dr. David Klein of Wilson Laboratories who supplied the brain extract employed.

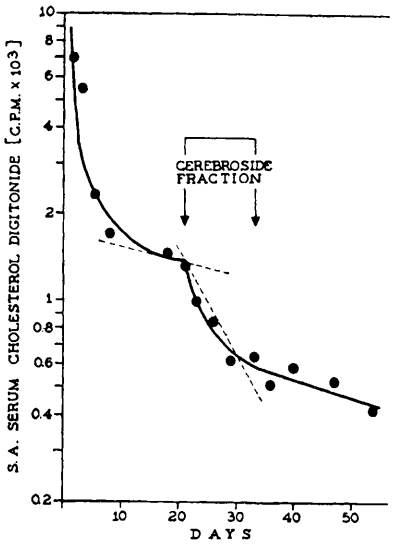


FIG. 1. Biological decay of $C^{14}$-cholesterol digitonide derived from serum of a chick which received a single intravenous injection of $C^{14}$-acetate at zero time. Dotted lines indicate the approximate slope of this decay curve immediately before and during period of administration of cerebroside fraction (from day 21 to 34).

cipitated quantitatively by digitonin by the method of Schoenheimer and Dam(8). The resulting precipitate was washed thrice with cold ethanol, once with ether:dioxane(1:1) and twice with ether, and the amount of sterol estimated from the digitonide which was dried for 30 minutes at 110°C and brought to constant weight in a desiccator. The total unsaponifiable matter was obtained gravimetrically by weighing the residue from an appropriate aliquot of the ether extract taken to dryness in a tared flask. The total unsaponifiable matter and digitonin precipitable sterol in the feces were then expressed as percent of dry weight of the fecal residue.

*Results.* In Fig. I the specific activity of the cholesterol digitonide precipitated from the serum lipids is plotted logarithmically against time. It can be seen that the rapid phase of the biological decay of labeled cholesterol is largely finished by the fifth day. After this the decay curve approaches a straight line asymptote, the slope of which is proportional to the turnover rate of the plasma cholesterol, provided the total plasma cholesterol level is constant(9). From previous experience we know that the cholesterol level of the plasma is but slightly influenced by the administration of brain extract when the bird is receiving a plain mash diet without oil or cholesterol supplement(10).

During the period of administration of the cerebroside fraction, there is a sharp increase in slope, the mean negative slope during this period being virtually twice that of the immediate pre-treatment period and 3 to 4 times that of the post-treatment period. The post-treatment slope cannot be said to differ from that anticipated had the more rapid decay period not intervened.

Fig. 2 illustrates that the same change in rate of turnover of serum cholesterol is induced in the dog during the 9 day period of administration of the extract. Serum cholesterol levels were obtained before and during administration of this cerebroside fraction by the Abell-Kendall technic(11) and the values during the control period ranged from 89-100 mg%, during the treatment period from 84-108 mg%. It was concluded that there was no significant change in the total serum cholesterol. As in the chicken, the turnover rate of the serum cholesterol did increase during the treatment period.

In Fig. 3 the decay curve for the $P^{32}$ labeled phospolipid is presented. The points approximate a straight line, though a slight increase in turnover rate of lipid phosphorus may be perceptible after the 4th day. There was no alteration in slope of the magnitude seen with the $C^{14}$ cholesterol. Again, there was no significant trend in the serum lipid phosphorus concentration, which varied between 6 and 7.1 mg%.

Fecal sterols were measured per unit dry weight of feces on 2 occasions, which may be considered representative of control and treatment periods. The results presented in Fig. 2 indicate that the total unsaponifiable matter per gram of weight (24 mg) was increased under treatment (35 mg) as was the weight of digitonin precipitable sterols per gram of dry weight of feces (6.4 mg to 14.0 mg). Thus, per unit of dry weight, the fecal sterols were increased by more than 2-fold. The specific activity of the digitonide on these 2 days was 27 and 44 counts per minute (corrected to infinite thickness). Thus, during

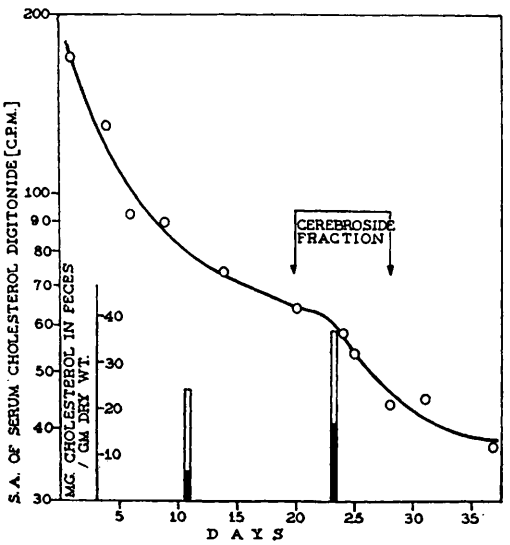


FIG. 2. Biological decay of $C^{14}$-cholesterol digitonide derived from serum of a dog which received a single intravenous injection of $C^{14}$-acetate at zero time. Total height of the bar represents milligrams of unsaponifiable matter per gram of dry weight of the feces; shaded portion represents milligrams of digitonin-precipitable sterol per gram of dry weight of feces on the days indicated. Cerebroside fraction was administered from day 20 to 29.

the feeding of the brain extract the specific activity of fecal sterols was 60% higher even though the specific activity of the serum cholesterol was, in fact, 30% lower than during the earlier period.

*Discussion.* These studies in the chick and the dog indicate that administration of this brain extract, rich in cerebrosides, will increase rate of turnover of the serum cholesterol, even in situations where no great change in total serum cholesterol obtains. There is ample evidence that an elevated total serum cholesterol will be reduced by this material(1,3), though no great change in serum cholesterol was expected in these experiments. Had a slight decrease occurred here, changes in the total specific activity of the serum cholesterol (S.A. $\times$ mg%) would have been even more dramatic. The turnover rate of phospholipid is but slightly influenced by the administration of the extract, there being no change comparable to that of the serum cholesterol. However, since the serum phospholipids include lecithin sphingomyelin and cephalins, an increased turnover of a minor component, such as sphingomyelin or a cephalin, cannot be excluded. With this reservation, we may conclude that the primary effect on the serum lipids is exerted upon the cholesterol rather than the phospholipids.

The nature of the brain extract effect seems to be an increase in turnover rate of the serum cholesterol. In the face of a constant serum level of cholesterol this implies an increased rate of removal from the serum of cholesterol higher in specific activity than that replacing it. Furthermore, this extract promotes an increased excretion of fecal sterols. Even more striking is the increase in their specific activity in the face of a falling specific activity of the serum cholesterol, whence it must be derived. These data strongly suggest a deflection of cholesterol from its entero-hepatic cycle. Similar observations on quantitative changes in fecal sterols have been made by Carroll *et al.*(12) who fed rats cerebrosidic fatty acids.

The mode of action of this extract is not yet entirely clear. A large percentage of the material remains in the intestinal tract to be excreted in the feces, thus increasing the bulk of the feces. The sterol seems to be intimately bound with this cerebroside residue, for it can be completely separated only with great difficulty. On the other hand, x-ray diffraction studies performed by the Physicochemical Research Division of the Lilly Research Laboratories, through Dr. R. E. Shipley, to whom we are deeply indebted for this kindness, have indicated that no mixed crystals can be distinguished when cholesterol and phrenosin are crystallized together in the same system productive of mixed cholesterol-beta-sitosterol crystals(13). It would appear that this cerebrosidic material somehow binds the

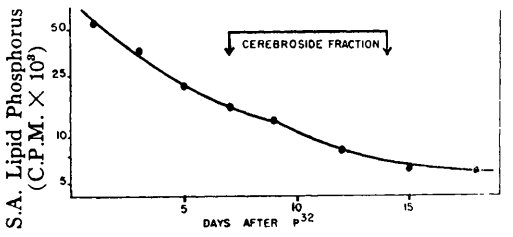


FIG. 3. Biological decay curve of $P^{32}$ phospholipids after a single intravenous injection of $Na_2HP^{32}O_4$ on 14th day of the experiment in Fig. 2, designated as day zero on the abscissa here.

intraintestinal cholesterol or otherwise inhibits its reabsorption into the portal system. This leads to an increase in the turnover rate of serum cholesterol and, if it is elevated, a reduction in its level.

*Summary.* In radioisotope studies in the chick and the dog, the turnover rate of the serum cholesterol, as measured by the biological decay of endogenously labeled $C^{14}$ cholesterol, was substantially increased by the feeding of a cerebroside-rich extract of mammalian brain. Much less influence was found on the turnover rate of $P^{32}$ labeled phospholipids. Some evidence is presented to suggest that the increased turnover rate of serum cholesterol is produced by an increased fecal excretion of endogenous cholesterol.

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## Effect of Gluco- and Mineralocorticoid Adrenal Steroids on Fluid and Electrolytes of Fasted Adrenalectomized Dogs.* (23504)

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Certain hormones of the adrenal cortex apparently are concerned with regulating the distribution of water and electrolytes between body compartments(1-11). Workers in this laboratory employing the potent steroid 2-methyl-9α-fluorohydrocortisone, which has both gluco- and mineralocorticoid activity demonstrated that adrenalectomized dogs verging on prostration from insufficiency could be restored to health and vigor within 48-60 hours despite withholding of food and water. Thus, dogs exhibiting severe symptoms evidently possess stores of water and salt in their cells and tissues adequate to effect apparent restoration of extracellular fluid and electrolyte to normal without necessitating additional supplies from exogenous sources(10-11). If cortical hormones are lacking such animals are unable to mobilize and freely shift these vital substances and death results. However, when the appropriate type of corticoid is administered i.v. to these critically ill dogs, large volumes of water, Na, Cl and K are withdrawn from unidentified stores and shifted to the extracellular and intravascular compartment. As a result, serum Na and Cl are elevated to normal, the inspissated blood becomes diluted and the marked accumulations of blood urea nitrogen and serum K decline to control values. These fluid and electrolyte transfers are accompanied by a

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