cation from a low external concentration is closely correlated with metabolic activity of the cells as gauged by O_2 consumption. It is possible, although the writers are not aware of substantiating data(13,14), that perhaps the adrenal glucocorticoids may be intimately associated with respiratory or more probably other tissue enzyme systems, which release the energy requisite for reducing the increased cellular osmolarity characteristic of the *adrenalectomized* dog exhibiting insufficiency or in a state of shock after subjection to stress.

Summary. Sensitivity of adrenalectomized dogs to stress induced by intestinal manipulation and metrazol is aparently due to inspissation of the extracellular compartment owing to passage of water into cells. Plasma volume and blood pressure decline, eventuating in circulatory failure. Adrenal glucocorticoids readily release the intracellular fluid and restore the fasted, stressed, dogs to normal. Glucocorticoids may be associated with tissue enzymes which release energy requisite to reduce the cellular hyperhydration characteristic of adrenalectomized dogs exhibiting insufficiency, or shock following stress.

1. Swingle, W. W., Parkins, W. M., Taylor, A. R., Hays, H. W., *Am. J. Physiol.,* 1936, v116, 438.

2. Swingle, W. W., Remington, J. W., Drill, V. A., Kleinberg, W., *ibid.,* 1942, 136, 567.

3. Swingle, W. W., Brannick, L. J., Osborn, M., Glenister, D., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 446.

4. Swingle, W. W., DaVanzo, J. P., Crossfield H. C., Glenister, D., Osborn, M., Wagle, G., *ibid.,* 1958, v99, 75.

5. Welt, L. G., Orloff, J., Kydd, D. M., Oltman, J. E., *J. Clin. Invest.,* 1950, v29, 935.

6. Hamburger, J., Mathé, G., *Physiologie Normale et Pathologique du Metabolisme de l'Eau,* Paris: Editions Medicales Flammarion, 1952.

7. Opie, E. L., *J. Exp. Med.,* 1949, v89, 185.

8. Robinson, J. R., *Biol. Rev.,* 1953, v28, 158.

9. Deyrup, Ingrith, *J. Gen. Physiol.,* 1952-53, v36, 739.

10. ———, *Am. J. Physiol.,* 1953, v175, 349.

11. Whittam, R., Davies, R. E., *Biochem. J.,* 1954, v56, 445.

12. Mudge, G., *Am. J. Physiol.,* 1951, v165, 113.

13. Bergen, J. R., Hunt, C. A., Hoagland, H., *ibid.,* 1952, v171, 624.

14. Crismon, J. M., Field, J., *ibid.,* 1940, v130, 231.

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Digestibility of Corn Germ as Factor in its Apparent Hypocholesterolemic Effects. (24720)

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Recent reports by Jones(1) and by Stamler(2,3) attribute protective effects against hypercholesterolemia and atherosclerotic lesions to whole corn germ when included in the diet of cockerels. This prompted us to investigate corn germ with the hope of isolating an active principle. Preliminary experiments confirmed apparent activity but raised a question regarding efficiency of digestion of the unground germ. If whole, unground germ is not well digested, comparisons may be made between groups of birds actually absorbing quite different amounts of oil from diets intended to be equivalent in oil content. Since it is standard practice not to include grit in the diet in such experiments, questions of digestibility are particularly pertinent. This report presents investigations of the effectiveness of corn germ and degree to which its oil content is available to the chick.

Methods. Cockerels were raised from birth on chick starter mash fed *ad lib.* They were divided into groups of 10 and placed on experimental diets when about 10 weeks of age and were kept on the diets for 5 weeks. At the end of the test period, blood was drawn for cholesterol determinations. These were run in duplicate on each sample by the method of Pearson(4). The birds were then sacrificed and lesions of the thoracic aorta and brachiocephalic arteries graded according to the scoring system of Tennent(5). Components of

TABLE I. Effects of Experimental Diets on Serum Cholesterol and Atherosclerotic Lesions.

Ration	Source of added lipid	Terminal mean serum cholesterol	Terminal mean atherosclerotic score	% lipid in feces	
Exp. 1					
I	10% refined corn oil	1650 ± 151*	2.08 ± .48*	4.5	
II	20% whole germ #1	834 ± 144	1.71 ± .60	18.5	
III	20% ground germ #1	1420 ± 170	3.21 ± .35	8.3	
IV	21.7% ground germ #2	1700 ± 115	2.17 ± .46	8.2	
Exp. 2					
V	10% refined corn oil	1551 ± 188	1.61 ± .55	3.1	3.3
VI	20% whole germ #1	1173 ± 159	1.94 ± .68	16.3	11.1
VII	20% ground germ #1	1806 ± 215	1.29 ± .45	6.0	6.3
VIII	20% whole germ #1 (1% granite grit in ration)	1306 ± 152	2.0 ± .55	2.4	4.1
IX	21.7% ground germ #2	1963 ± 159	1.95 ± .47	6.3	6.0
X	19.4% ground germ #3	1324 ± 117	1.90 ± .43	6.1	6.2
XI	None	1168 ± 162	1.40 ± .46	3.0	2.9
Exp. 3					
XIIa	10% refined corn oil	2356 ± 257	1.61 ± .39	2.6	
b	*Idem*	2052 ± 178	1.70 ± .39	3.1	
XIIIa	19.4% whole germ #3	962 ± 42	.69 ± .20	17.7	16.6
b	*Idem*	1086 ± 174	.89 ± .39	18.7	17.8
XIVa	19.4% ground germ #3	1975 ± 181	.81 ± .38	5.3	
b	*Idem*	1838 ± 183	1.22 ± .34	5.2	

* ± S.E.

the experimental diets in each case except ration XI were chick starter mash, sucrose, 1% cholesterol and a source of corn lipid. Diets were adjusted to contain 15% protein and 10% added corn lipid. Diet XI differed in that no corn lipid was added. The germ used was prepared by the wet-milling process and was consequently relatively unbroken. Three different lots of germ were compared in the experiments. The sterol content of lot #1 of germ was 0.51% as determined by analysis of petroleum ether extracts made before and after acid hydrolysis. The refined corn oil used in the investigations contained 0.96% sterol. Total lipids in corn germ and feces were determined by exhaustive extraction with 30-60°C petroleum ether in a Soxhlet apparatus of dried and ground samples. There were slight differences in details of first 3 experiments. Cockerels in Exp. 1 were Leghorns of the Hyline Cross and those in Exp. 2 and 3 were of H & N Cross. Ground Wayne Chick Starter* containing 20% protein and 4% fat was used in Exp. 1 and 2. To parallel more closely the rations used by Stamler, chick starter containing 20% protein and 3% fat, manufactured by Kahn Bros. of Chicago, was used in Exp. 3. Exp. 3 differed from the others also in that each ration was fed to 2 different groups of 10 birds in an attempt to assess replicability of results. Exp. 4 was limited to a test of digestibility of whole germ. Fourteen cockerels of 14 weeks of age which had never received grit were placed on a ration similar to that used in the other experiments and containing 20% wet-milled corn germ. After a week on the diet, a record was made of feed consumption for a 48-hour period. Care was taken to recover all uneaten food including that which was dropped on the cage floors by the birds. All fecal droppings were collected during the 48-hour period and weighed after drying. The entire collection was ground and mixed and aliquots were then removed for lipid determination.

Results. Results of feeding experiments may be seen in Table I. In each case feeding of whole germ gave significantly lower serum cholesterol values than were obtained when ground germ was fed. Further, cholesterol values obtained when whole germ was fed are lower in each case than when corn oil was fed although the difference is not statistically sig-

* Manufactured by Allied Mills, Chicago, Ill.

TABLE II. Recovery of Ingested Fat in Feces (Exp. 4).

Total corn germ consumed in 48 hr (4460 g feed × 20% germ)	892 g
Total corn fat consumed in 48 hr (892 g germ × 43.84% fat)	392
Total fat in 48 hr fecal collection (1920 g feces × 19.75%* fat)	389

* Individual determinations were 20.23, 19.62 and 19.41% fat.

nificant in Exp. 2. The picture is less clear when atherosclerotic lesion scores are considered. Only in Exp. 3 are consistent and significant differences in lesion scores seen. Here the lesions were less severe with a high degree of significance when the whole germ was used as a source of corn lipids (Group XIII) than when corn oil was used (Group XII). The difference between lesion scores for Groups XII and XIV is not significant at the 95% level.

An indication of differences between lots of ground germ is seen in serum cholesterol values for Groups IX and X. Their difference meets tests of significance.

It is important to the interpretation of results that similar blood cholesterol values were seen in group receiving no added oil (Group XI), and the group receiving whole corn germ (Group VI). This is to be expected if little or none of the whole germ is digested. Some measure of the digestion of the germ is to be seen in the fat content of the recovered feces. Little fat was found in the feces when lipid was added to the diet as liquid oil; much when whole germ was used. When ground germ was the source of lipid, fecal fat was low to intermediate in amount. The use of grit in the diet apparently allowed complete digestion of the germ since a low fat content was found in the feces of Group VIII in spite of the fact that the germ was added whole.

Further reinforcing the concept that utilization of the whole germ is very poor in the absence of grit is the observation that large amounts of apparently discrete germ were seen in the fecal matter when such diets were fed. Table II reports a more complete attempt to measure digestion of whole germ added to the diet. The total amount of fat present in the feces was measured over a 48-hour period and was found to nearly equal the fat in the corn germ ingested in the same interval. The nearly exact agreement recorded must certainly have been fortuitous since corrections must be made for unsaponifiables and for incomplete absorption of fat under even the best conditions. Application of a correction based on lipid content of the feces of chicks receiving the same diet but without corn germ (such as diets V, XI) would seem to be the most accurate way of making such allowances. When this is done, the recovery of oil from the ingested corn germ is at least 81%.

Discussion. The results certainly show that a large part of the observed protective effect of whole corn germ is an artifact of incomplete digestion of the germ. At the same time there remain some hints that germ may have a real effect. Among these are: 1) differences between germ lots 2 and 3 as seen in Groups IX and X, 2) failure of germ plus grit (Group VIII) to give as high cholesterol values as ground germ (Group VII), thus suggesting a possible deterioration of an active principle on exposure of the ground germ to air, and 3) lower atherosclerotic lesion scores seen when ground germ was fed rather than refined corn oil in Exp. 3. Of these hints, only the difference between corn germ lots 2 and 3 meets tests of significance.

Cholesterol values in general are higher than those reported by other workers in similar experiments(1,6). Lesion scores are correspondingly high. The reasons for these deviations are not known. Replicability is seen to be good as tested in Exp. 3.

Summary. Digestion of whole germ by chicks not receiving grit is poor. This explains to a large extent the apparent effects of whole corn germ in maintaining low cholesterol levels and preventing atherosclerotic lesions.

1. Jones, R. J., Reiss, O. K., Huffman, S., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 88.

2. Pick, R., Stamler, J., Katz, L. N., *Circulation,* 1957, v16, 513.

3. Stamler, J., Pick, R., Katz, L. N., *Fed. Proc.,* 1958, v17, 155.

4. Pearson, S., Stern, S., McGavack, T. H., *Anal. Chem.*, 1953, v25, 813.

5. Tennent, D. M., Siegel, H., Kuron, G. W., Ott, W. H., Mushett, C. W., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 679.

6. Katz, L. N., Stamler, J., Pick, R., *Nutrition and Atherosclerosis,* 1958, Lea and Febiger, Philadelphia, 61-75.

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Effect of Spirolactone SC 8109 on Renal Function in Normal Human Subjects.* (24721)

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A natriuretic effect of synthetic steroids (spirolactones) has recently been shown in the rat, dog(1), and man(2). Kagawa, *et al.* (1) demonstrated that these spirolactones are virtually ineffective in the absence of mineralocorticoid and that effects upon urinary electrolyte excretion are constant so long as a constant ratio of spirolactone/mineralocorticoid is maintained. Similar observations have been reported by Liddle(2) in the human subject. Sodium diuresis has been induced in normal human beings on salt restriction and in patients with aldosterone-secreting adrenal adenoma despite increasing levels of urinary aldosterone(2,3,4). Although Salassa, Mattox, and Power(4) and Liddle(2) have reported that the 24-hour clearance of endogenous creatinine is unchanged in the sodium diuresis induced by spirolactones in man, the literature contains no precise measurements of renal hemodynamics during administration of these substances. The purpose of this study has been to examine more carefully renal function in the normal human subject under the influence of endogenous aldosterone with and without administration of spirolactone.

Materials and methods. The substance used was 3-(3-oxo-17*beta*-hydroxy-19-nor-4-androsten-17*alpha*-yl)propionic acid *gamma*-lactone known by the code name, SC 8109.‡ Drug administration was at a dosage level of 50 mg each 3 hours beginning at 6:00 to 8:00 a.m. on the day preceding study and continuing until the beginning of the clearance periods. Normal, young, male volunteers were fed a diet containing 20 to 30 meq of sodium, under the supervision of a dietitian. Water and potassium were allowed *ad libitum*. Following 5 to 7 days of sodium restriction, the subjects were studied to determine inulin, para-aminohippurate (PAH) and free water clearances and sodium, potassium and chloride excretion rates. Sodium restriction was continued until the 24-hour urinary excretion rate was in the range observed prior to the first study, then a second set of clearance studies was done. In 3 subjects the control studies were obtained prior to the test, while the remaining 2 were studied in the reverse order. Subjects were studied between 8 a.m. and noon following a 16-hour fast. The men were placed in bed with head elevated to 45° and were not allowed to smoke. A water diuresis was induced by oral administration of 20 ml of water/kilogram of body weight and diuresis was sustained by replacement of urine volume plus estimated unmeasured water loss every 15 minutes. Four control and 4 treatment clearance periods of 15 to 20 min. duration were obtained according to accepted technics of timing and collection of samples(5). Sustaining solutions were administered in a 0.9% solution of sodium chloride at the rate of 3.8 to 4.0 ml/min as controlled by a constant rate infusion pump. Following each experiment, the data were analyzed by Barnard's

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