and also after the carbohydrate diet had been decreased. The blood was obtained through the kindness of Dr. Sherrill.

DETERMINATIONS OF CALCIUM IN BLOOD OF DIABETES BEFORE AND AFTER REDUCED CARBOHYDRATE DIET

	Before			*After*	
Patient	Date	Calcium	Sugar	Date	Calcium	Sugar
McS	3-28-22	5.9	0.367	5-18-22	6.4	0.157
Miss L.	- -22	7.0	0.375	4- 3-22	7.1	0.112
F. R.	3- 1-22	9.0	0.577	5-18-22	5.6	0.129
R.	2-22-22	10.5	0.441	3- 7-22	8.6	0.159
B.	2-16-22	5.7	0.652	2-27-22	6.4	0.341
R.	2-11-22	8.1	.....	3-11-22	8.1	0.166

Calcium determination of the blood of a patient, Mrs. A. G., with osteomalacia.

February 8, 1922. (Had been treated with calcium lactate) 10.1 mg.
February 16, 1922. 9.4 mg.
February 24, 1922. 11.5 mg.
March 1, 1922. 11.6 mg. in uterine blood.

CONCLUSIONS

In some patients with systemic furunculosis, if the blood is tested at the time an acute boil is present the calcium content is found to be below normal.

The calcium is below normal in many young children who have pneumonia.

Not all of the patients with tetany had low calcium.

The placing of diabetic patients on a low carbohydrate diet did not affect the blood calcium in any definite manner.

One patient with osteomalacia had a normal amount of calcium in the blood.

154 (2114)

The genesis of gall stones in the dog.

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In dogs permanently intubated for the collection of bile, gall stones not infrequently develop despite the absence of infection, stasis and gall bladder activity. The character of the stones has already been discussed.[1] They are always discrete to begin with,

[1] Rous, Peyton and McMaster, P. D., *Jour. Exper. Med.*, 1921, xxxiv, 47.

scattered upon the glass and rubber wall of the collecting tube. What determines this punctate localization? In certain instances, of almost pure calcium carbonate concretions, the answer is plain. These form in the midst of organic debris, not infrequently around bits of talc from the tube surface. In other cases minute, rounded, pigmented particles from the bile lodge upon the tube wall, and stone formation takes place upon these as nuclei. To trace the source of such particles and their significance we have made day to day studies of the sediment from sterile 24 hour specimens of bile centrifuged on removal from collecting balloons devoid of air. Also we have followed the early stages of calculus formation in the collecting tubes of the same animals.

The bile was found to yield formed elements identical with those later recovered from the tube system and from the interior of stones forming upon the walls of the latter. The nature and the amount of the sediment vary with the condition of the animal. For a day after the operation whereby intubation is effected it may consist merely of mucus, which later is seldom met with. Usually one obtains from the specimen of the second 24 hours after operation and perhaps more abundantly from that of the third, a slight brown deposit, made up, as the microscope shows, of minute, highly refractile, translucent, yellow-brown granules. The shape of these tends to be spherical, but is rendered various by the partial merging of the spheres. They fracture radially on pressure into rosettes, are anisotropic, and fail to lose this character or their shape when heated to 100° C. The refractile material of which they are mainly composed is insoluble in water, alcohol, ether, chloroform, or acetone, but dissolves readily in chloroform after treatment with acid, as also in a dilute watery solution of hydrochloric acid, leaving the brown pigment and a mucous "shadow" behind. They are colored deep blue with Nile blue sulphate but are unaffected by the stains for neutral fats, and are Gram-negative. Such granules serve as nuclei for the deposition of calcium carbonate and calcium bilirubinate, as further study of the bile has shown. In the secretion of the third to fifth day after operation, but usually not later, they are present but encrusted with a deposit of more or less pigmented crystals composed of a mixture of the salts mentioned. Thereafter this crystalline matter alone is to be found, unless there occurs some liver disturbance, when a shower of the brown granules

may again appear in the bile and crystalline deposition again takes place upon them. We have observed such a sequence of events after poisoning with chlorform or toluylenediamine, after biliary obstruction of some days' duration, and following intravenous injection of a concentrated solution of calcium chloride. The majority of the stones forming upon the canula and collecting tube within the animal have such granules as their nuclei.

Whence come these granules? They suggest in size and shape the "bile thrombi" observed under pathological conditions within liver tissue. By digesting the tissue with trypsin such "thrombi" can be obtained separately, and their characters compared with those of the granules,—from which they are then found to differ in many respects, notably in being isotropic. Yet our observations leave no doubt that "thrombi" identical with those in the liver do sometimes appear in the bile. Whether they have any relation to gall stones has not been determined.

The dogs studied were losing all of their bile. May this not have been a factor in the cholelithiasis? That it was not a primary factor was shown by interpolating small glass tubes into duct systems left with intestinal connection undisturbed. In some of the animals thus treated, stones of the characteristic sort formed upon the glass. This being true, why were they never found in the ducts, or, more especially, in the gall bladder of healthy animals? Foreign bodies left in the latter viscus under aseptic conditions fail to bring about the development of stones.[2] The ducts, elaborating, as they do, a secretion of their own,[3] and provided with a musculature, may well be able to rid themselves of particulate matter. But the shortcomings in this connection of the gall bladder are strikingly proven by the frequent occurrence in it of a shreddy, cellular debris which, if present in a glass-rubber system, would almost infallibly lead to a formation of calculi. The cause for the absence of stones from the gall bladder is to be found in the change in the reaction of the bile which this organ effects. It acts to render the secretion acid, even acid to litmus, as others have noted before us.[4] The P_H of hepatic duct bile, as determined electrometrically, ranges between 7.5 and

[2] Mignot, R., *Arch. gen. de Med.*, 1898, I, VIII Ser., T. X., 129.

[3] Rous, Peyton and McMaster, P. D., *Jour. Exper. Med.*, 1921, xxxiv, 75.

[4] Okada, S., *Jour. Phys.*, 1915-16, l, 114; Neilson, N. M., and Meyer, K. F., *Jour. Infect. Dis.*, 1921, xxviii, 130.

8.5, while that of gall-bladder bile from the same animal may fall as low as 5.18. Test tube experiments show that when liver bile containing a crystalline sediment of the sort out of which stones form is gradually brought to the average reaction of bladder bile, by the addition of N/10 HCl, or 3 per cent. acetic acid or concentrated lactic acid, the sediment goes into solution.

From these observations the inference seems justified that many sorts of liver derangement conduce to the presence, in bile that is sterile, of potential nuclei for calculus formation. But the normal gall bladder effects precisely the sort of change in the fluid that renders stone formation upon such nuclei impossible. The organ does not merely concentrate[5] and store the bile, it alters it so that it can be stored safely. In the lack of such alteration, as when the gall bladder fails to function normally, it is not surprising that stones should form.

There are facts in the literature which indicate that the principles here set forth apply to other species besides the dog. The reaction of rabbit bile from gall bladders rendered abnormal by typhoid infection is not acid like that from the healthy organ but has the same alkalinity as liver bile.[4] And in such gall bladders stones composed of calcium salts are regularly found if the animal lives long.[6] This cholelithiasis has heretofore been attributed to infection and inflammation, factors which are, at the most, accessory judging from the evidence here presented. We are now attempting to determine whether the change in reaction has any relation to the state of the cholesterin in human bile.

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5 Rous, Peyton, and McMaster, P. D., *Jour. Exp. Med.*, 1921, xxxiv, 47.

6 Meyer, K. F., Neilson, N. M., and Feusier, M. L., *Jour. Infect. Dis*, 1921, xxviii, 76.