the creatine diet died as a result of specific poisoning. The analytical values for the young adult and the old animals showed no significant difference attributable to age. The figures have, therefore, been combined in the results presented.

It has previously been found (Chanutin[3]) that massive doses of creatine over shorter periods may increase the content of creatine in rats. Our results indicate that more prolonged feeding of lower doses, which in total exceed the quantities fed over the shorter periods, does not lead to an augmentation in creatine content of the tissues we have examined.

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Are Neutral Fat and Lecithin Present in Gall Bladder Bile?

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Since the time of Strecker[1] chemists who have worked with bile have appreciated the difficulty of separating lipoids from bile. The lipoid constituents as given by the text books at the present time are merely repetitions of the early figures given by Strecker, Hammarsten,[2] von Gorup-Besanez,[3] and others. These constituents are given as fatty acids, soaps, phosphatides, fat, and cholesterol. An examination of this early literature does not yield satisfactory evidence for the presence of either neutral fat or lecithin in bile.

Fresh bile may be extracted in 2 ways. One is to deproteinize by means of alcohol, then dry the protein-free bile and dissolve the residue in absolute alcohol, filtering off the inorganic salts, that are precipitated, and finally pouring the alcoholic solution into a large volume of anhydrous ethyl ether. This is the method used by Hammarsten.

The second method is to extract directly with ethyl and petroleum ether in the presence of some alcohol. In this manner a very persistent emulsion is formed that requires time to break. In either

3 Chanutin, A., and Beard, H. H., *J. Biol. Chem.*, 1928, **78**, 167; Chanutin, A., and Silvette, H., *J. Biol. Chem.*, 1928, **80**, 589; Chanutin, Alfred, *J. Biol. Chem.*, 1930, **89**, 765.

1 Strecker, *Compt. Rend.*, 1861, **52**, 1270.

2 Hammarsten, *Nova Acta Reg. Soc. Upsala*, 1893, June 15.

3 Von Gorup-Besanez, *Prager Vrtl. jahrshrift für prakt. Pharm.*, 1851, III, 86.

method a sticky molasses-like substance is obtained on evaporating the ether, which does not look like fat and contains bile salts, fatty acid, phosphorus, sulphur and nitrogen containing compounds and non-saponifiable material. After as thorough an extraction as can be carried out in this manner, saponification of the non-ether soluble residue with strong alkali will permit the extraction of much fatty acid that could not be extracted in the first place.

When 2,500 cc. of beef gall bladder bile is dried in air, and then taken up in alcohol and precipitated in ether, the ether takes up 11.45 gm. of material or 0.458%.

Suspecting that the drying of bile and solution in alcohol might hydrolyze the fats, bile was extracted directly using a modification of the Roese-Gottlieb[4] method for fat extraction from milk. This method proved very difficult on account of the persistent emulsions that were formed. A typical experiment of this kind will be described. To 800 cc. of fresh hog bile placed in a tall cylinder, were added 200 cc. of alcohol, and 1,000 cc. of a mixture of equal parts by volume of ethyl and petroleum ether. The cylinder was shaken vigorously and then allowed to stand for several days. In time this mixture separated into 4 layers. On the bottom was a yellow precipitate of protein nature, above this a clear brownish colored watery solution of bile, above this a cloudy gray emulsion of bile and ether in which was suspended some of the protein precipitate, and finally above this a layer of clear ether. Both the clear layer and the emulsion were removed separately. The clear watery solution of bile was again extracted 3 or 4 times in the same way. The clear ether extracts were united and the emulsions were combined and evaporated to dryness. The residue from this was redissolved in ether and filtered, when a clear solution was usually obtained. All the ether extracts were now united, evaporated to dryness and the fatty material recovered.

The ether-soluble residue from this extraction was a brown colored, waxy solid containing much soft crystalline material and was 0.48% of the original bile (480 mg. per 100 cc.) On saponification and reextraction of the material extractable in ether from an alkaline solution, 80% of the material was recovered as non-saponifiable matter and of this 78% was cholesterol by the Liebermann-Burchard method. The remainder was probably higher alcohols. Hence, from 100 parts of this fatty substance 20% was fatty acid, 62% cholesterol and 18% non-saponifiable matter other than cholesterol.

[4] Roese-Gottlieb, *Z. Nahr Genüss m.*, 1905, **9**, 531; *A. O. A. C. Methods*, 1925, 262.

On saponification of a sample of the whole bile by means of potassium hydroxide followed by extraction with petroleum ether, the non-saponifiable matter was .28% and the saponifiable matter 2.82%. Hence the direct extraction of fatty material from bile yields only a very small part of the total fatty acids that are present in bile.

The mixed gall bladder bile of at least 30 different steers was subjected to this extraction. From 2,000 cc. of this bile, 10.95 gm. of material were obtained, of which one gram was non-saponifiable material. This 10.95 gm. of material was saponified with sodium hydroxide for 24 hours, cooled, acidified with hydrochloric acid and allowed to stand in the cold for 12 hours. The fatty acids were filtered off and the filter washed twice with cold water. This clear filtrate was treated with benzoyl chloride as in the Schotten-Baumann method for alcohol detection as described by Mulliken.[5] Thirty-five milligrams of precipitate was obtained that in no way resembled glycerol mono- or tri-benzoate, and which gave no odor of acrolein when heated with potassium acid sulphate.

Under the above conditions as little as 12 mg. of glycerol in dilute solution can be easily identified by this method. By the acrolein method alone, as little as 5 mg. can be detected. Hence in this fatty material there must be less than 50 mg. of neutral fat or lecithin or less than 0.5% of the possible amount.

The acrolein test was further made on fatty extracts from dog, beef, and hog bile in amounts of from one to 2 gm. of ether soluble extract. In no case could the odor of acrolein be detected.

On one large sample of fat (bile) an attempt was made to separate glycerol by the formation of sodium glyceride.[6] This gave a negative result. On 2 trials with 100 cc. of dog bile it was attempted to isolate glycerol directly by extracting the fat, saponifying and acidifying this fat, and then removing all interfering substances from the watery solution. On evaporating this watery solution nothing that reacted like glycerol could be detected.

In conclusion, these experiments conducted on the mixed gall bladder bile of the ox, the hog, and the dog indicate that neutral fat and lecithin are either absent from the gall bladder bile of these animals or is present in very minute quantities.

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[5] Mulliken, *Identification of Pure Organic Compounds*, 1904, Vol. 1, 169.

[6] Heinrich, *Bull. Trdsk. Kemi Farmaci 09 Terapi*, 1917, **12**. Abstracted in *Chem. Abst.*, 1917, **11**, 216.