the distilled metal to flow in a thin stream first through a column of dilute nitric acid, then dilute sulphuric, to dissolve out all lead and tin, finally washed and dried. All apparatus was most carefully cleansed, and when necessary moisture-free and proved to be air-tight.

UNIVERSITY OF NORTH CAROLINA,
July, 1897.

ON THE DETERMINATION OF FAT AND CASEIN IN FECES.

BY HERMAN POOLE.

Received August 24, 1897.

HAVING been called upon to make some examinations of feces from children in connection with the clinical experiments on the Prof. Gaertner mother-milk now being carried on with satisfactory results at the German Polyclinic on Seventh Street, New York City, I began by looking up what had been done on the subject previously. To my surprise I found nothing at all which would give even a fairly approximate idea of the percentage of fat and casein. After consulting all the authorities I could find, I saw that I must work out a new or a fuller method than any previously used.

The problem to solve was this: A certain child takes per day a definite quantity of milk of known composition. It digests a portion of this and passes off the balance undigested. This milk contains fat and casein. How much fat and how much casein pass through undigested, and from this how much of the milk is assimilated?

Of course, one of the first books suggested to an American would be Flint's Physiology, since Flint has done considerable work on this kind of material several years ago. An examination of his work, however, showed that the methods he used and the special objects he had in view were entirely unsuited and foreign to the work I intended. He operated on the feces of adults, and more particularly to discover and extract the unknown and undescribed constituents rather than to actually make accurate determinations of the common and known ones. Besides, the chemical methods in use in his day are to a considerable extent supplanted by more accurate and modern methods.

Papers have been published and articles written on the analyses of feces for certain purposes by Rubner, Camerer, Prausnitz, Mayer, and Constanidini in the *Zeitschrift für Biologie* in most of the volumes since 1879, and by Malfatti and Strumpell,[1] but none of these in any way outline methods which would be used in my investigation. The fullest account given is that by Prausnitz,[2] in which he sums up what has been done and gives the result of the most recent work up to that time.

But all these methods are insufficient. not carrying the analysis far enough. Neither attempts to separate the fat or the casein in a state approaching freedom from other allied or commixed substances. All give the results of imperfect separations.

The fat determinations are all made by drying the material at 100° and then extracting with ether. This ether extract is evaporated; the residue is weighed and calculated as fat. This would be a very simple and easy method, but, unfortunately, it is not correct and can give only misleading deductions. In addition to the fatty matter, ether dissolves cholesterol and some of the bile and other products. The proportion of cholesterol is not constant, varying from twenty-five to seventy-five per cent.; the amount contained varies from day to day with the same child, and even varies with the successive passages. Still greater then must be the variation with different children. The proportion of the other substances is small and may be neglected ordinarily. It will be easily seen, therefore, that deductions based on such determinations must be faulty.

In the determination of casein no satisfactory method has yet been devised that I know of. The authorities cited have taken the ether extract, the water extract, and the alcohol extract; the residue was then treated by some method for determining the nitrogen and it was given as such, no attempt being made to separate the nitrogen of the food, digested or undigested, from that of extraneous bodies which might be present. This method is even more faulty than the fat determination. It shows the amount of nitrogen passing off through this channel but gives no idea as to the form in which it occurs.

1 *Konig's Chem. der Mensch. Nahrung und Genuss*, I, 37 and 46.

2 *Ztschr. für Biol.*, *1894*, pp. 328 *et seq.*

The insoluble residue, after the three menstrua above mentioned have been used, contains the insoluble casein, the insoluble fatty acid compounds (usually lime, alumina, and iron as bases) and the broken down epithelium cells gathered from the whole length of the intestinal tract. This waste epithelium is at times quite an important factor and is always present in an appreciable degree as can be seen by a microscopic examination. The fatty acid compounds, of course, contain no nitrogen. The three menstrua fortunately remove most of the numerous nitrogenous substances present in the original material, leaving only a small quantity not in the epithelium cells or in the casein; so that it may be considered that the remaining undissolved mass consists substantially of the last two.

The problem then becomes one of separating the cholesterol from the fat and the casein from the epithelium cells and other foreign matter.

First, separation of the fat and cholesterol. The ether extract containing these two and the coloring matter from the bile is sometimes yellow, sometimes brown or greenish. It always contains some substances insoluble, or difficultly soluble, in alcoholic potash, which is used for saponification and solution of the fat and cholesterol.

The ether extract is evaporated nearly dry at 100° C. and then heated at 110° C. till dry. This heating to 100° is necessary, since, although ether is used as a solvent, this ether always contains a little water, sometimes more than will be driven off at 100°. The residue is then saponified with alcoholic potash, which usually leaves only a small portion undissolved. When thoroughly saponified, water is added and the whole boiled to expel the alcohol. Then more water is added, if needed, and the solution filtered. The filtrate should be clear and opalescent, but is usually colored. Now pour the filtrate into a separatory globe or cylinder and agitate thoroughly with an equal bulk of ether. Allow to stand and separate. Draw off the liquids separately and again treat the heavier one with ether as before. A further treatment with ether usually reveals only slight traces of cholesterol and need not be made. The liquid thus freed from cholesterol is now evaporated nearly dry and dissolved in water. It contains the fat acids, which may be determined by any of the usual methods, and multiplication by the proper factor will give the amount of butter fat originally contained in the feces as such.

Secondly, the separation of the casein from the other substances. This is a difficult separation. None of the strong solvents, alkalies, strong acids, etc., can be used as they destroy both, or while attacking one, attack the other. Neutral salts are not satisfactory for dissolving insoluble casein since large quantities of liquid must be used, and even then a second treatment with the same solvent will usually dissolve more.

The method which I finally adopted, while not entirely satisfactory, still gives results far nearer the truth than any of the published ones and, at least for the purpose in view, comparable results.

The feces are extracted in succession by ether, water, and alcohol, and then dried. The residue is digested for several hours (usually over night) in a mixture of thirty parts hydrochloric acid and seventy parts water at a temperature of about 50° C. This dissolves the casein and leaves the epithelium débris and other substances. The fatty acid compounds are of course decomposed, but do not influence the result at all. Hydrochloric acid is more satisfactory than the neutral salts and gives fairly concordant results on duplicate trials. After cooling sufficiently, the liquid is filtered off, evaporated, and the nitrogen determined by the Kjeldahl method. From this the quantity of casein is calculated and the percentage determined.

In a careful, thorough analysis the quantity of fatty acids in the insoluble soap would of course be determined, but with my investigation this is not necessary, as they usually represent digested or partially changed fats, and I am looking only for the undigested portions.

My results of course are much lower than would be obtained by the old method, but I claim they are nearer the truth and perfectly comparable among themselves. They certainly do show more nearly the composition of the material examined and furnish a better guide as to the workings of the food on the child and the child on the food.

No percentage results are given in this paper as no work has yet been done on perfectly healthy children, and such work is needed as control.

The investigation will be pursued and if possible more satisfactory methods devised. Any suggestions from chemists having experience in this line will be thankfully received.

THE PRINCIPAL AMID OF SUGAR-CANE.

BY EDMUND C. SHOREY.

Received October 4, 1897.

IT has been more than forty years since Lawes and Gilbert pointed out that in plants used as feed for stock, part of the nitrogen exists in the amid form. Since then several schemes of analysis have been devised by which to determine the amounts of different forms of nitrogen in plants, and numbers of analyses have been published in accordance with these schemes, so that it has come to be generally accepted by chemists that a part of the nitrogen of all plants, in the growing stage at least, is in the amid form. E. Schulze, who has done more than anyone else to advance our knowledge of the forms in which nitrogen exists in plants, states that the amid compounds vary with different plants, and also with the age and condition of the plant.

In 1892 I determined the total and albuminoid nitrogen content of a number of samples of mature sugar-cane. In the samples then examined I found the albuminoid to be about ninety per cent. of the total nitrogen. Analyses made later of another and less mature variety of cane, showed this non-albuminoid nitrogen to be sometimes as high as twenty-five per cent. of the total nitrogen. The albuminoid nitrogen was determined by precipitating with cupric hydroxide, taking special precautions to prevent any decomposition of the albuminoids by preliminary heating. No attempt was made at that time to determine the character of the non-albuminoid nitrogen.

In January, 1894, a paper was read by W. Maxwell before the Louisiana Sugar Planter's Association on "Organic Solids not Sugar in Cane Juice." The matter of this paper was subsequently issued as a bulletin by the Louisiana Experiment Station. In this paper attention was drawn to the fact already noticed, that all plants contain at some period part of their nitrogen in the amid form: a fact well known to chemists, but apparently overlooked by sugar manufacturers. After giving a number of analyses of cane juice, in which the difference