THE CHEMICAL DIAGNOSIS OF STEATORRHOEA

BY

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The term "steatorrhea" was introduced by Kuntzmann in 1824 to describe specimens of faeces in which globules of fat were visible to the naked eye. It has long been known that there may be an excess of faecal fat which is not visibly obvious, and the term is now generally used to include such specimens. Apart from those cases of gross steatorrhea where the condition is at once obvious, the diagnosis of this condition is entirely dependent on chemical methods of analysis, and the interpretation of results is made very difficult by the problem of individual variation and by vague definitions and bad nomenclature. The main difficulties of interpretation are these:

(a) The bowel habits of different individuals, and of the same individual at different times, vary considerably. The excreted mass is inhomogeneous, which immediately introduces all the errors of sampling; and even in normal subjects the amount of fat excreted per day (no matter how the results are expressed) is known to vary within wide limits.

(b) The term steatorrhea means, literally, an excess of fat in the faeces, but this in turn may mean one or other of two quite different things: either an abnormally high percentage of fat in the dried specimen or an abnormally high daily excretion of faecal fat. These two quantities are not the same, and, what is much more important, they can vary independently.

(c) The expression "fat balance" is commonly used to describe the correlation between the amount of fat excreted per day over a given period and the dietary fat intake, when this intake is fixed at a given value, generally in the region of 50–75 g. per day. A balance experiment is a well-defined scientific procedure used in the investigation of total metabolism, and the so-called "fat balance" is not a balance experiment in any sense of the term. This most unfortunate nomenclature not only is the cause of much confusion but makes it almost impossible to interpret many of the experimental results found in the literature. However, since the expression is so thoroughly established in general use, it will be retained here—under protest.

(d) The fat which is excreted in the faeces has little relationship, if any, to the dietary fat in normal animals. Hill and Bloor (1922) showed conclusively that the quantity of faecal fat was reasonably constant in any given animal (their experiments were carried out on cats) and was virtually independent of the amount of fat in the diet. These findings have been confirmed by many others, and particularly, in normal human subjects, by Annegers, Boutwell, and Ivy (1948), whose paper on the subject leaves no room for doubt.

(e) Sperry (1929) showed that a substantial part, estimated at up to 40%, of the faecal fat was derived from the bacteria in the lower intestine and was presumably the result of bacterial synthesis. It is also well known that gut-sterilizing drugs such as sulphaguanidine and terramycin bring about a considerable drop in the percentage of fat in the faeces. More recently, Sammons, Vaughan, and Frazer (1956) have isolated a strain of *Streptococcus faecalis* which can synthesize large amounts of long-chain fatty acids *in vitro*.

(f) And, lastly, there is evidence that the bowel mucosa can actively secrete fat into the lumen (Lewis and Partin, 1954).

Establishing a diagnosis of steatorrhea now goes far beyond the recognition of coeliac disease in children and idiopathic steatorrhea in adults. It is a matter of some importance in many disorders, such as the post-gastrectomy syndrome, the malabsorption syndrome, certain disturbances of calcium and of iron metabolism, and other equally ill-defined conditions, and certain lines of research into these disorders involve the investigation of a possible state of steatorrhea.

The data presented here are the results of fat-balance experiments dealt with by this department over a period of two years. The object of this survey was to decide how far chemical analysis could assist in establishing or rejecting a diagnosis of steatorrhea, and, at the same time, to decide between the relative merits of a fat analysis of a random specimen of faeces and the results obtained by a fat balance. No attempt has been made to correlate the clinical diagnosis with the chemical results. All cases of gross steatorrhea, where the diagnosis was not in dispute, have been excluded from this survey, which is concerned only with those cases where the question of steatorrhea had been raised by the clinician and material sent to the biochemist in an attempt to provide the answer.

Subjects and Method

All the subjects were in-patients admitted for a variety of reasons, and, with one exception, all were adults. As stated above, all cases of severe steatorrhea where the diagnosis could be made by inspection of the faeces have been omitted from consideration, since they do not constitute any diagnostic problem which requires reference to a biochemist. The subjects included two separate series of cases which were admitted for special investigations—namely, a series of post-gastrectomy cases and a series of cases of osteoporosis. The other cases were the usual miscellaneous collection to be found in any general hospital and in which a state of steatorrhea was at least possible.

The patients were placed on a mixed diet containing 70–75 g. of fat per day, and a few days' equilibration was allowed before collection of specimens was started. The bowel wash-out procedure was *not* used, as experience had shown that it invariably resulted in a state of complete constipation for several days. Markers were not used, but emptying of the bowel was as complete as could reasonably be expected. The usual precautions were taken to avoid the use of purgatives (especially paraffin oil), contamination with barium sulphate, carrying out experiments on any patient suffering from diarrhoea, or experiments on any patients undergoing a therapeutic course of gut-sterilizing drugs.

The length of the "balance period" varied from three to seven days. This is much less than the optimal period, and, as Annegers *et al.* (1948) have so clearly shown, the shorter the balance period, the greater the normal range of variation of results. But in a general hospital one is clearly limited both by the ward and by the laboratory facilities, and the object of the exercise is simply to confirm or reject a diagnosis of steatorrhea.

In all, 138 patients were studied and 231 fat balances were carried out. In 66 of these patients a fat balance was carried out more than once, and this immediately raised the question of whether each fat balance should be considered separately, or whether all the fat balances done on any one patient should be combined into one

result—the latter especially in those cases where two or more fat balances had been carried out in immediate succession. There are several arguments for and against, but it was finally decided to consider each fat balance separately, since there was often a long period of time between the balances, many of the subjects had started some form of therapy between the balance periods, and, in some cases, after the initial fat balance, gluten was added to the diet to find out if it would induce a steatorrhoea. In such cases, both the clinician and the biochemist are equally interested in any change in fat excretion which may occur.

Estimation of Faecal Fat

The entire faecal mass was evaporated to dryness on the steam bath. The dried mass was then stirred up with 100 ml. ethyl alcohol containing 0.5% (v/v) concentrated hydrochloric acid in order to liberate free fatty acids from soaps, and again evaporated to dryness. When dry, the material was transferred to a large mortar and thoroughly ground up. 2 g. of the powder was weighed out, placed in a smaller mortar, and re-ground with a little silver sand which had previously been extracted with ether. This mixture was transferred quantitatively to a Soxhlet thimble and extracted with diethyl ether for four to five hours in a Soxhlet apparatus. The ether was evaporated off from the extract, and the residue was dissolved in petroleum ether (b.p. 40–60° C.). The petroleum-ether extract was filtered through paper into a tared flask, the solvent evaporated off, the flask cooled and dried in a desiccator, and weighed. The results are expressed as g. fat per 100 g. dried faeces, commonly referred to as the “percentage faecal fat.” The total weight of the dried faecal mass was also determined, and, knowing the number of days over which the balance extended, the daily fat excretion was calculated.

This method determines the total ether- and petroleum-ether-soluble lipid, and includes steroids, steroid esters, carotenoids, higher alcohols, etc. It is often stated that certain volatile (? fatty) acids are lost during the drying process. Any loss from this source is unlikely to be very great, since the main part of the drying is carried out before addition of acid, at which time the fatty acids are present in the form of non-volatile soaps. Any small error due to loss during the second evaporation is partly counterbalanced by the extreme efficiency of extraction in the Soxhlet apparatus compared with “wet” extraction. It might also be added that the b.p. of the 4-carbon compound *n*-butyric acid is 163° C.

Standards of Reference

In spite of the wide individual variation, it seems to be generally accepted that normal adult faeces usually contain 10–25 g. fat per 100 g. dried specimen (10–25% fat). This is the range quoted by Stewart and Dunlop (1958) and by King and Wootton (1956). Cantarow and Trumper (1955) give a range of 15–25% of the dry weight, while Varley (1958) quotes no lower limit but agrees with an upper limit of 25% fat in the dried specimen. Certainly, from the analyses recorded in this survey, the lower limit is frequently less than 10% of the dry weight, but, so far as the diagnosis of steatorrhoea is concerned, only the upper limit is important. For the purposes of classification of results, any specimen containing 25% of fat, or more, will be regarded as “abnormal.”

The weight of fat excreted per day by the normal adult is a much more doubtful quantity. Most estimates have been quoted in terms of the “percentage fat absorption”—an expression of very questionable validity—but when expressed in this way all authors seem to be agreed that the “percentage absorption” in the normal individual should be not less than 90%, and several suggest a value of 95%, or more. It is implied (but seldom stated) that the 10%, or the 5%, of fat which is not absorbed is excreted in the faeces. On this basis, if the daily fat intake be fixed at 70 g., and the percentage fat absorption is 95%, the daily fat excretion should be 3.5 g.

There are few data of the actual amount of fat excreted per day by the normal adult. Those figures which appear to be most reliable are summarized in Table I.

TABLE I.—Daily Fat Excretion by Normal Healthy Adults

Reference	No. of Sub-jects	No. of Experi-ments	Daily Fat Intake	Total Fat Excreted (g. per Day)
Cooke <i>et al.</i> (1946)	41	50	50 g.	2.5 ± 2.0
Wollaeger <i>et al.</i> (1947)	11	11	102 g.	4.1 ± 0.5
Annegers <i>et al.</i> (1948)	40	220	93–168 g.	3.9 (s.d. = 1.0)

The normal upper limits are taken as 4.5 g., 4.6 g., and 5.9 g. respectively (mean = 5.0 g.).

For the purposes of classifying results, any specimens corresponding to an excretion of 5 g. of fat, or more, per day will be regarded as abnormal. On an intake of 70–75 g. of fat per day, such an excretion would correspond to a “percentage fat absorption” of 93%.

It is inevitable that any classification of data based on such arbitrary limits of normality must lead to a number of borderline cases; but it was found that a subdivision of analytical results, based on the standards of reference quoted above, gave remarkably few borderline cases.

The fat analysis of a single random specimen of faeces has the great advantage that the result depends solely on analytical accuracy; the disadvantage is that it gives no indication in most cases of the daily excretion of fat unless the result is either very high or very low. The daily excretion of fat can be obtained only from the analysis of the total output of faeces, which carries the disadvantage that it introduces a second variable—the completeness of the collection of the specimen, or, put in another way, this second variable is the average dry weight of faeces excreted per day. The introduction of this second variable is extremely important, and it is invariably ignored. For example, a patient may have a normal percentage of fat in the faeces, yet if the bulk of the dried specimen is greater than normal, the daily fat excretion may be above the accepted normal upper limit. Since these two quantities (the percentage of fat in the faeces and the total fat excreted per day) can vary independently, there are four possible combinations of results, and for the purposes of classification these will be referred to as groups A, B, C, and D.

Group A.—Faecal fat less than 25% of the dry weight; fat excretion less than 5 g. per day.

Group B.—Faecal fat less than 25% of the dry weight; fat excretion of 5 g. per day, or more.

Group C.—Faecal fat of 25%, or more, of the dry weight; fat excretion less than 5 g. per day.

Group D.—Faecal fat of 25%, or more, of the dry weight; fat excretion of 5 g. per day, or more.

The subjects in group A are clearly not cases of steatorrhoea on either count. The subjects in group D were suffering from steatorrhoea at the time when the experiment was carried out, no matter which of the two definitions of steatorrhoea is used. The cases in groups B and C may be either normal or abnormal.

Results

Since the upper limits of the "normal" percentage of faecal fat and of the "normal" daily fat excretion have been decided in advance, it is pointless to quote any standard deviations, etc., and only the mean values for each group need be considered. When classified according to the limits described above, the results were those set out in Table II.

TABLE II.—*Fat Excretion of Patients Suspected of Having Steatorrhoea. 138 patients. Total Number of Fat Balances, 231*

Group:	A	B	C	D
No. of Balances:	159	24	25	23
Percentage fat in the dried specimen	13.4%	19.7%	30.2%	29.6%
Daily excretion of fat	2.1 g.	8.2 g.	3.1 g.	7.2 g.
Dry weight of faeces excreted per day	16.9 g.	40.5 g.	10.5 g.	24.4 g.

Group A

The number of cases which could be regarded as bordering on the other three groups was surprisingly small. Of the 159 cases, 141 (89%) had a percentage fat less than 20% of the dry weight, and 147 (92%) had a total fat excretion of less than 4 g. per day. In 132 cases (83%) the percentage fat was less than 20% of the dry weight, and the fat excretion was less than 4 g. per day, so that any possibility of steatorrhoea could be completely ruled out. Indeed, in only four of the 159 cases did the percentage fat lie between 20 and 25% of the dry weight and the daily fat excretion between 4 and 5 g. in the same balance experiment, and only in these four cases could a diagnosis of steatorrhoea be seriously considered.

In this group the average dry weight of faecal material passed per day was 16.9 g., the extreme limits being 1 and 70 g. In 133 (84%) of these 159 cases the average dry weight of faecal excretion lay between 5 and 25 g. per day. The "normal" dry weight of total solids excreted per day was found by Wollaeger, Comfort, and Osterberg (1947) to be 27.6 ± 2.2 g., with an observed range of 13.6 to 39.1 g. (11 subjects). Diao and Johnston (1957) found a mean value of 20.9 g., with an observed range of 14.3 to 26.5 g. (9 subjects).

The cases in this group cannot be regarded as "controls," nor are they a series of normal healthy individuals, since in every case there was some clinical ground for suspecting that fat excretion might be abnormal. On the other hand, since it is quite clear that 97% of the subjects were *not* suffering from steatorrhoea, they can be used as a basis for comparison with the other three groups.

The lower "normal" limit for the percentage of fat in the dried faeces is generally quoted as 10 to 15%. Of the 159 analyses in this group, 42 (26%) had a percentage faecal fat less than 10% of the dry weight—and that in a group where there was at least a clinical suspicion of the possibility of steatorrhoea. It is suggested that a lower limit of even 10% of fat in the dried specimen is much too high, and should be reduced to 5% fat.

Group B

Although in this group the average percentage of fat (19.7%) in the dried faeces is much higher than the average for the cases in group A, the figure is still well within the accepted limits of normality. But of these 24 cases, only 11 had a faecal fat less than 20% of the dry weight (compared with 141 out of 159 such cases in group A). More than half the cases in this group had a "high normal" percentage of faecal fat—that is, between 20 and 25% fat.

The average daily fat excretion (8.2 g.) was as high as 15.3 g. in one case, and in almost half the cases (11 out of 24) the daily fat excretion was greater than 7 g.

The abnormally high daily fat excretion in this group is directly due to the large faecal mass. The average weight of dry faecal material excreted per day was 40.5 g., which is more than double the corresponding figure for the subjects in group A. In group B the observed range was 21 to 68 g. There is good evidence for the conclusion that these cases are, in fact, cases of steatorrhoea (see below).

Group C

The average percentage of faecal fat (30.2%) in this group is well above the accepted upper limit of normality; but if this were the sole criterion used, many of the cases in group C would have to be regarded as borderline. In 8 of the 25 cases the percentage faecal fat lay between 25 and 27% of the dry weight.

The average daily fat excretion (3.1 g.) was little greater than the corresponding value for the cases of group A, and in 18 of the 25 cases the excretion of fat was less than 4 g. per day. The finding of a normal daily fat excretion in group C is directly due to the small daily faecal mass. The average weight of dry faecal material passed per day was only 10.5 g. In spite of the abnormally high percentage fat, there are good reasons for believing that the cases in this group are not suffering from steatorrhoea (see below).

Group D

This group has been confined to cases in which a diagnosis of steatorrhoea had to be made on analytical grounds. As stated above, all cases of gross steatorrhoea were excluded from this series, so that the mean values recorded in Table II do not appear to be very striking. It will be noticed that the average percentage faecal fat is not very different from the corresponding figure for the cases in group C, and that the daily fat excretion is not very different from the corresponding figure for the cases in group B.

Repeated Fat Balances

In 66 of the patients the fat balance was repeated at least once. In 45 (67%) of these cases agreement was satisfactory and there was no doubt about the group into which the result should be placed. In 10 cases agreement was very close, and Case 59 (Table III) shows an example of a typical borderline case where two different balances fell into separate groups. In 7 cases agreement was poor, but the change in fat excretion could be ascribed to therapy—for example, the decreasing steatorrhoea in Case 103. In the remaining 4 cases the lack of agreement was due to a considerable change in the bulk of the faecal material excreted—for example, Case 128.

The last case quoted in Table III (Case 29) will be referred to later in the text.

TABLE III.—*Examples of Repeated Fat Balances*

Case	Balance	Per-centage Fat	Fat Excreted per Day	Dry Weight of Faeces per Day	Group
59	1	23.1	5.8 g.	25 g.	B
	2	26.1	5.2 „	20 „	D
103	1	28.5	12.4 „	44 „	D
	2	23.9	15.3 „	64 „	B
	3	14.9	9.1 „	61 „	B
	4	10.3	5.6 „	54 „	B
128	1	25.6	7.3 „	28 „	D
	2	29.5	2.4 „	8 „	C
29	1	1.0	0.7 „	70 „	A
	2	2.6	1.4 „	56 „	A
	3	2.5	0.9 „	35 „	A
	4	5.4	1.1 „	20 „	A

Discussion

The great preponderance of results falling into group A shows that a diagnosis of steatorrhoea is proposed much oftener than it can be substantiated. On the other hand, it is very important in many cases to exclude the possibility of steatorrhoea. If the percentage of cases falling into group A appears unduly high, it must also be remembered that in cases of gross steatorrhoea there is no virtue in chemical analyses to support a diagnosis which is clearly obvious, and, even if the clinician is following the effects of therapy, inspection of the faecal mass in these cases is generally sufficient for the purpose. It must be admitted, however, that there are occasions when the clinician persists in attempting to demonstrate a state of steatorrhoea where the condition does not exist. Case 29 (Table III) is an example. This was a case of very severe osteomalacia with many very interesting biochemical findings—but steatorrhoea was not one of them.

In this survey the most interesting cases are those of groups B and C. Either, or both, could be regarded as cases of steatorrhoea depending on the definition.

Group B

If steatorrhoea be defined as the excretion of more than 5 g. of fat per day on an average diet, then all the patients in this group must be regarded as cases of steatorrhoea. But they are placed in this category only by virtue of the large mass of faecal residue excreted per day. It could be argued that any normal individual might land in this group simply by excreting a sufficiently large quantity of faeces. If the percentage faecal fat is 13.4% (the mean of the cases in group A) a normal individual would require to pass 38 g. of faecal solids per day in order to qualify for group B and a label of steatorrhoea. But the excretion of 38 g. of faecal solids per day is more than twice the average for the cases of group A, and of the 159 cases in group A, only 6 excreted more than 38 g. of dry faecal residue per day. This cannot therefore be accepted as a logical argument, and other considerations lead to the conclusion that the subjects in group B are indeed cases of steatorrhoea.

The average daily fat excretion of the cases of group B was 8.2 g., which is four times the average for the cases of group A, and, as it so happened, this figure was also higher than the corresponding figure for the undoubted cases of steatorrhoea in group D (although if all the cases of gross steatorrhoea had been included the figures

for the cases of group D would have been very different). The percentage faecal fat was within normal limits, but more than half the cases (13 out of 24) had values in the upper normal range of 20 to 25% fat. This compares very badly with 18 out of 159 corresponding cases in group A. The association between steatorrhoea and the passage of bulky faeces has long been recognized. The average weight of dry faecal material passed by the cases in group B was almost $2\frac{1}{2}$ times the corresponding figure for the cases of group A, and it was also considerably higher than the value for the established cases of steatorrhoea in group D. As a general rule, the total daily amount of any substance excreted from the body is of more fundamental importance than the concentration of that particular substance in the excretion. This has been well established in studies of urinary constituents, and no doubt applies also to faeces, although one would not care to push the analogy too far. The "percentage faecal fat" is strictly analogous, however, to a concentration.

All these considerations point strongly to the conclusion that the cases of group B are cases of steatorrhoea.

Group C

The cases of group C qualify for a diagnosis of steatorrhoea only because of the abnormally high concentration of fat in the dried faeces.

In spite of the high concentration of fat in the dried specimen, the daily output of fat is low, and is nearer to the corresponding value for the cases of group A (2.1 g.) than to the agreed upper normal limit (5 g.). The average dry weight of faecal solids (10.5 g.) was much less than the figure for the cases of group A, and the most obvious explanation is that the diet was deficient in indigestible residue. But since all four groups of patients were on approximately the same hospital diet, this cannot be the reason. Another more likely possibility is that breakdown and absorption of carbohydrate and protein was more complete than normal. This possibility remains to be explored. Whatever the mechanism, it is clear that addition to the diet of a greater amount of indigestible material would result in more bulky faeces and a fall in the observed percentage of faecal fat without affecting the total daily output of fat. If the average dry weight of faecal solids were increased from 10.5 to 16.9 g. per day by the addition of *non-fatty* residue in the diet, the percentage faecal fat of the cases in group C would drop from 30.2% to 18.8% and would then be well within the normally accepted limits. The daily output of fat would remain at 3.1 g. There are therefore good grounds for the belief that the cases of group C are *not* cases of steatorrhoea.

While these arguments and conclusions are strongly in favour of the determination of the daily fat excretion as the better criterion of a diagnosis of steatorrhoea (and this seems to be the generally accepted view), the determination of the percentage faecal fat of a random specimen is by no means a useless procedure.

Of the total of 47 cases of steatorrhoea in groups B and D, only one case had a percentage faecal fat less than 14%—the actual figure being 10.3% fat. This patient was passing an average of 54 g. of faecal solids per day. Therefore, if the percentage of faecal fat is less than 14% a state of steatorrhoea is most unlikely unless the faecal mass is abnormally bulky. If the

figure is less than 10% of the dry weight a diagnosis of steatorrhoea can be confidently excluded.

Of the 159 experiments in group A, the percentage faecal fat was less than 10% in 44 cases (27%). In those 44 cases a diagnosis of steatorrhoea could have been ruled out by the analysis of a random specimen of faeces, with the saving of a great amount of time and work both in the ward and in the laboratory. In a further 48 cases (30%) the percentage faecal fat lay between 10 and 14%, and in these, also, a diagnosis of steatorrhoea could have been excluded, with considerable confidence on the result of the analysis of a random specimen. Put in another way, 92 of the 231 fat balances carried out in this series (that is, 40%) were simply a waste of time.

Summary

The results of 231 fat balances in 138 patients suspected of having steatorrhoea have been critically examined. From a study of the literature it appears that any percentage faecal fat over 25%, or any daily excretion of fat greater than 5 g., is probably abnormal—the latter figure being more significant than the former. On this basis the 231 fat balances have been divided into four groups, and the significance of the findings of each group has been discussed.

The total weight of dry faecal material excreted per day is an important figure, and account must be taken of it when examining the results of any fat balance. It should also be recorded in the biochemical report.

All cases where the daily excretion of fat is abnormally high should be regarded as cases of steatorrhoea, irrespective of the percentage faecal fat. Cases where the percentage faecal fat is abnormally high but where the daily output of fat is within normal limits (that is, where the daily faecal mass is abnormally low) should not be diagnosed as steatorrhoea.

Chemical analysis of a random specimen of faeces is a very useful procedure in excluding a possible diagnosis of steatorrhoea. If the percentage faecal fat is less than 10% of the dry weight, steatorrhoea is excluded. If the value lies between 10 and 14% a diagnosis of steatorrhoea is most improbable unless the patient is also passing very bulky faeces. The great advantages of the analysis of a random specimen of faeces are (1) the saving of time and work both in the ward and in the laboratory, and (2) the fact that it can be successfully used as a screening test on out-patients.

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FREEZE-DRIED B.C.G. VACCINE

STABILITY OF THE VACCINE UNDER DIFFERENT CONDITIONS OF STORAGE AND PERSISTENCE OF TUBERCULIN SENSITIVITY IN SCHOOLCHILDREN AFTER VACCINATION

SECOND REPORT TO THE MEDICAL RESEARCH COUNCIL BY THEIR COMMITTEE ON THE STANDARDIZATION OF FREEZE-DRIED B.C.G. VACCINE*

During recent years freeze-dried B.C.G. vaccine has been developed in a number of countries. In Britain the vaccine is prepared by Glaxo Laboratories Ltd., and the method employed has been described by Ungar, Farmer, and Muggleton (1956). Freeze-dried B.C.G. vaccine has advantages over the liquid preparation in that it can be fully tested in the laboratory before it is issued for use, and its stability gives it a considerably longer life. It was decided, however, before using the vaccine extensively in this country to investigate the consistency of the viable count of a number of routinely prepared batches, and also to compare its ability to produce tuberculin sensitivity with that of the Danish liquid vaccine, which has now been shown to confer substantial protection against tuberculosis for at least six and a half years (Medical Research Council, 1956, 1959). A previous report, giving the results of a preliminary investigation (Medical Research Council, 1958), showed that the range of variation in viable count was no greater with the British dried vaccine than it was with the Danish liquid vaccine, and that the conversion rates produced by the batches of dried vaccine used in this study, although slightly less than with the liquid, were nevertheless satisfactory.

In the preliminary investigation, the dried vaccine used had been stored continuously in the refrigerator since its preparation, and it was found that periods of up to 10 months' storage did not affect its ability to produce a satisfactory conversion rate. It was considered important, however, to make further investigations, by both laboratory and clinical methods, of the stability of the vaccine at different temperatures and after different periods of storage in order to obtain information which would serve as a basis for recommendations relating to storage. It was also considered advisable to investigate the persistence of tuberculin sensitivity after vaccination. In the new studies, therefore, tuberculin tests were made both at one to

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