*A Study of the Variations in the Secretion of Hydrochloric Acid in the Gastric Contents of Mice and Rats as compared with the Human Subject, in Cancer.**

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In March, 1905, Dr. Benjamin Moore, in collaboration with Messrs. Alexander, Kelly, and Roaf, communicated a paper† to this Society on the "Absence or Marked Diminution of Free Hydrochloric Acid in the Gastric Contents in Malignant Disease of Organs other than the Stomach." The conclusions arrived at were based on the analyses of test meals given to human subjects.

Later in 1905, Dr. Willcox, of St. Mary's Hospital, published a paper on the same subject in the 'Lancet,'‡ and his results appeared to confirm generally those of Dr. Moore. Other workers, among whom Dr. Morton Palmer§ may specially be mentioned, followed on the same lines, and the matter attracted considerable attention among physiologists.

It occurred to us that in connection with the extensive researches in progress at the laboratories of the Imperial Cancer Research Fund, there was a mass of material ready to hand in the large number of mice under observation there. Accordingly we approached the Director of the Laboratories, Dr. Bashford, on the subject, with the suggestion that we should endeavour to control observations on the human subject by determining the physiologically active hydrochloric acid both in the stomachs of normal mice and in those of mice the subjects of transplanted tumour growth. Dr. Bashford most kindly acquiesced and offered to place any necessary materials at our entire disposal.

The only chemical work that had been done in connection with mice was by Dr. Cramer,‖ in the laboratory of the Imperial Cancer Research Fund, who, in 1905, made some estimations of the total acidity in the stomach

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† 'Roy. Soc. Proc.,' B, vol. 76, 1905, p. 138.

‡ 'Lancet,' June 10, 1905, p. 1566.

§ Guy's Hospital Reports, vol. 60, 1906, p. 181.

‖ 'Biochemisches Centralblatt,' vol. 4, p. 65, June, 1905.

contents of mice inoculated with alveolar carcinoma (Jensen), and had found that there was, apparently, no decrease in this acidity as compared with normal mice.

We commenced our experiments in December, 1905, and have continued them with comparatively little interruption up to the present time. At the beginning of this year (1908) we were fortunate in obtaining a grant from the Imperial Cancer Research Fund, which, by enabling us to retain the services of a skilled assistant, rendered possible the completion of our work, in the course of which, during the last two and a-half years, we have had the opportunity of examining over 1000 stomachs of normal and of "cancer" mice.

Our primary object was to ascertain whether the suggested absence or marked diminution of free hydrochloric acid in the stomachs of human beings suffering from cancer occurred also in mice suffering from the same disease, whether spontaneously arising or the result of experimental transplantation. Obviously the *method* to be adopted for the determination of the hydrochloric acid was an important consideration, and after careful study of the various processes used by other investigators in similar work, we finally determined to employ Volhard's method for the volumetric estimation of chlorides, the principle of which is based on the precipitation of the chloride by an excess of standard silver nitrate in the presence of free nitric acid and the subsequent determination of the excess of silver nitrate by means of ammonium thiocyanate, using iron alum as an indicator.

This method was ingeniously adapted by Lüttke,* and further simplified by Willcox, for the purpose of estimating hydrochloric acid in gastric contents, and we have followed these adaptations with such further modifications as seemed to us necessary for our particular purposes.

The special advantage of Volhard's process, apart from its accuracy, is that the hydrochloric acid, free or combined, is precipitated as chloride of silver in presence of free nitric acid. There can therefore be no question as to the particular acid estimated, a point which has been raised, although in our opinion needlessly, in connection with the Mörner Sjöqvist method of estimating free and combined hydrochloric acid (see later).

Briefly, the *modus operandi* of the method as employed by us has been as follows:—The stomachs are counted, weighed, crushed, and macerated in water for about 12 hours, and the mixture made up to a known volume and filtered. The examination of the filtrate is then carried out in the following manner:—

(*a*) The *total chlorides* (free, inorganically and organically combined hydrochloric acid) are estimated by Volhard's method in an aliquot part.

* 'Deutsch. Med. Woch.,' 1891, p. 1325.

(*b*) The *inorganic chlorides* are similarly estimated in another aliquot part after evaporation to dryness on a water-bath and charring the residue at a gentle heat.

(*c*) The *total acidity* is determined in another aliquot part by titration with standard sodium hydrate, using phenolphthalëin as an indicator.

From these three determinations may be deduced:—

1. Physiologically active hydrochloric acid = $(a-b)$.
2. Organic acids = $(c-(a-b))$.

All of these various constituents are, for convenience, expressed in terms of hydrochloric acid.*

The term *physiologically active hydrochloric acid* is intended to include both the free acid and that combined with proteids and nitrogenous organic bases, since, as originally pointed out by Lüttke in 1891,† and more recently emphasised by Willcox, much misconception has arisen in the use of the terms "free" and "combined" as applied to hydrochloric acid in the gastric contents, and it has been erroneously held that the presence of the free acid is of more importance than the organically combined acid. But inasmuch as the latter may have been free a short time before its estimation, the exclusive determination of "free" hydrochloric acid cannot but lead to fallacious conclusions.

With this introductory explanation, we may now consider the summary of the results which we have obtained.

(1) *Experiments on Mice made without Reference to Time of Digestion* (*Series* 1 *to* 5, *Mice over* 12 *months old*).

The first five series of experiments (December 19, 1905, to May 7, 1906) were made without reference to time of digestion, not because we had not

* In the estimations of total and inorganic chlorides in mice stomachs, 20 c.c. to 50 c.c. of aqueous extract were taken, according as the extract had been made up to 100 c.c. or 250 c.c. It might appear probable that a source of error would arise in the charring of the residue, due to partial volatilisation of inorganic chlorides; we tested this point rigidly by repeating the estimations in a very large number of instances, but our experience was that there was practically no error, the difference in the titration numbers being no greater than that usually found in closely agreeing estimations. The presence of the charred matter does not interfere in the end-reaction when the quantity of chlorides present is comparatively large, but is liable to cloak the reaction when the amount is small. To overcome this difficulty we found it was only necessary to add a somewhat larger excess of standard silver nitrate solution, so that when titrating back with ammonium thiocyanate the obscuring effect of the charred matter was annulled by the extra amount of white silver thiocyanate in suspension. By adopting these modifications and by using N/50 or even N/100 instead of N/10 standard solutions, we were able to determine the chlorides in a single stomach with accuracy and comparative ease.

† *Loc. cit.*

fully considered the possible importance of this point, but more with a view of noting, as a preliminary step, whether under conditions of ordinary feeding any appreciable difference could be demonstrated in the amount of physiologically active hydrochloric acid present in the stomachs of normal mice as compared with that present in the stomachs of mice with transplanted tumours.

The results obtained indicated, somewhat to our surprise, an increase rather than a decrease of hydrochloric acid in stomachs of mice with transplanted tumours, as the following summary (Table A) shows:—

Table A.*—Percentage of Physiologically Active Hydrochloric Acid in the Gastric Contents of Normal Mice, and of Mice with Transplanted Tumours, without Reference to Time of Digestion.

Series.	Date.	Normal mice.		Mice with non-ulcerated tumours.		Mice with ulcerated tumours.	
		No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.
1	Dec. 19, 1905	6	0·0382	4	0·2169	2	0·2321
2	Jan. 4, 1906	19	0·1841	17	0·1003	2	0·1494
3	Jan. 31, 1906	40	0·1127	27	0·1522	10	0·1711
4	Mar. 12, 1906	45	0·1081	37	0·1133	14	0·1085
5	May 7, 1906	40	0·0930	61	0·2549	4	0·1740
Average		150	0·1121	146	0·1815	32	0·1465

* The tumours in these series were all alveolar carcinoma (Jensen), and varied in weight from 0·2 to 10 grammes.

Owing to the small weight of the individual stomachs (averaging about 0·75 gramme), we did not at first see our way to determining the chlorides in a single stomach, though later on (see footnote, p. 446) we were able to accomplish this with accuracy. We therefore took batches of stomachs varying in number from 6 to 60 with a view to arriving at a general comparative average. From Table A it will be seen that in the normal mice the physiologically active hydrochloric acid varied from 0·0382 to 0·1841 per cent. with an average of 0·1121 per cent.; in the mice with non-ulcerated tumours it varied from 0·1003 to 0·2549 per cent. with an average of 0·1815 per cent. and in the mice with ulcerated tumours it varied between 0·1085 and 0·2321 per cent., with an average of 0·1465 per cent.

Thus under exactly similar conditions but without reference to time of digestion, the mice being simply taken from their cages, in which there was

always food, and killed at once, the mice with transplanted tumours showed an average greater proportion of physiologically active hydrochloric acid in the gastric contents, with a less variation between the extremes, than was the case with the normal mice.

It is only right to state that in the case of the mice with tumours referred to in Table A, although they were fed and killed as above stated, the stomachs were not sent to us at once, but kept, in an ice safe in glass tubes with cotton wool stoppers, for periods varying from a few days to a few weeks; the stomachs of the normal mice, however, killed as controls, were sent in a fresh condition. It was obvious that this procedure was not altogether satisfactory and in all the other experiments we arranged to have the stomachs sent to us perfectly fresh, so that there was the least possible delay between the time of killing and the commencement of their analysis. It must be further stated, however, that the above experiments are as perfectly reliable as the later experiments with fresh stomachs, which tended in all respects to confirm these first results.

(2) *Experiments made on Mice and Rats with Special Reference to Time of Digestion.*

It was obvious, however, that a definite period of digestion would be likely to give a more strictly comparable series of results, and we selected in the first instance a period of one hour. The mice, whether normal or with transplanted tumours, were put in cages overnight without food, were fed next morning and killed one hour after feeding.* Series 6 to 10 were conducted in this manner and a summary of results is given in Table B. The mice in this case, as in the previous series, were over twelve months old. The results obtained again appeared to emphasise, even more strongly, the fact that there was an average increase in the secretion of hydrochloric acid in the stomachs of mice with transplanted tumours as compared with normal mice during a period of digestion of one hour.

Thus, as against an average of 0·1488 per cent. in normal mice, we found an average of 0·1627 per cent. in mice with non-ulcerated tumours and of 0·2100 per cent. in mice with ulcerated tumours.

But a difficulty in these, as also in the previous experiments, was that although in the whole of the 10 series, with two exceptions only, a more or less considerable increase of hydrochloric acid was indicated in the case of the mice with transplanted tumours, the actual percentages of hydrochloric acid in each class varied nevertheless somewhat largely *inter se*, and more especially in the normal stomachs.

* The food consisted of bread soaked in water, oats, and canary seed.

Table B.—Percentage of Physiologically Active Hydrochloric Acid in the Gastric Contents of Normal Mice, and of Mice with Transplanted Tumours. Period of digestion, 1 hour.

Series.	Date.	Normal mice.		Mice with non-ulcerated tumours.		Mice with ulcerated tumours.	
		No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.
6	May 30, 1906	32	0·1374	35	0·1393	21	0·2439
7	June 21, 1906	16	0·1992	15	0·1569	11	0·1898
8	Jan. 15, 1907	40	0·1188	50	0·1660	20	0·1856
9	Feb. 4, 1907	30	0·1322	45	0·1509*		
10	Feb. 28, 1907	26	0·1974	38	0·1964		
Average		144	0·1488	183	0·1627	52	0·2100

* These mice had been inoculated from Tumour "No. 27" (adeno-carcinoma); all the rest from alveolar carcinoma (Jensen). The weights of their tumours varied practically over the same range as given in note to Table A.

We had already considered the question as to whether it might be better to analyse the gastric contents apart from the very thin stomach walls, and we made a number of experiments in this direction. The following typical instance of such results in Series 8 (Table B) shows that the presence of the walls modifies but very slightly the results which would have been obtained for walls and contents taken together, and affects the comparison practically not at all.

——	Normal.	Non-ulcerated.	Ulcerated.
	HCl per cent.	HCl per cent.	HCl per cent.
Contents	0·1221	0·1783	0·1940
Walls	0·1061	0·1052	0·1357
Contents and walls	0·1188	0·1660	0·1856

We therefore decided to continue the experiments, as we had commenced them, by taking the walls and contents together.

We therefore next endeavoured by a further series of experiments to determine, if possible, the usual period of digestion of normal mice, so that this being done, we could then make further comparisons with mice with transplanted tumours under exactly similar conditions as to feeding and periods of digestion. We selected the following periods: half an hour, one hour (with mice under three months), and one hour and a-half.

Table C gives a summary of the results which we obtained and we have also included previous results for the sake of general comparison.

We found in the half hour's digestion, for mice over twelve months, the average in Series 12 was 0·1129 (10 stomachs), with a variation from the low figure 0·0611 to 0·2037 per cent., and in Series 16 the figures vary from 0·1243 to 0·1765 per cent., with an average of 0·1451 (7 stomachs). On the other hand, for mice under three months we obtained an average of 0·1881 per cent., with a variation from 0·1438 to 0·2167 per cent. (13 stomachs). The general average for all half hour experiments is 0·1530.

It seems to us, with this very considerable variation, scarcely worth while to continue the investigation of the half hour period.

Taking the one-hour period of digestion, the average results are more uniform, although there are considerable variations. The average for mice over twelve months (144 stomachs) being 0·1488, while that for mice under three months (55 stomachs) is 0·1445.

As regards the one-and-a-half-hour period, the average results were also very uniform and the variation distinctly less, the average for mice over twelve months being 0·1826 per cent., while that for mice under three months is 0·1866 per cent, the extreme variations being 0·1515 and 0·2643 per cent.

From these figures it is evident that no definite period of digestion is indicated from these experiments; all that can be said is that some mice have attained a maximum at half an hour, and most have attained a maximum at an hour and a-half, while at one hour a large proportion have attained a maximum. The explanation of this variation undoubtedly lies in the fact that mice are in the habit of continually feeding, and that therefore the intervals of rest from feeding are comparatively rare.

We next made further experiments as to the secretion of hydrochloric acid by mice with transplanted tumours during periods of digestion of one hour and one and a-half hours respectively. A summary of the results obtained is given in Table D, in which are also included our previous experiments, for the purpose of general comparison.

It will be seen from an inspection of Table D that the results for one hour's digestion of mice with non-ulcerated tumours (Series 6 to 10) gave an average of 0·1627 per cent. (183 stomachs), with a variation from 0·1393 to 0·1964 per cent.; while mice with ulcerated tumours (Series 6 to 8) gave an average of 0·2100 per cent., with a variation of 0·1856 to 0·2439 per cent.; the total average for these 235 stomachs was 0·1732 per cent., all the mice being over twelve months.

For mice under three months with transplanted tumours (Series 25 and 27), four gave an average of 0·1856 per cent. and seven an average of

0·1569 per cent., with a total average of 0·1673 and a variation from 0·1421 to 0·2102 per cent. during one hour's digestion.

With regard to the one-and-a-half hour period, 12 mice with transplanted tumours gave an average of 0·1605 per cent. (Series 22), 11 gave an average of 0·1260 per cent. (Series 23), and 11 gave an average of 0·1246 per cent. (Series 24), the general average being 0·1377, with a variation between 0·1013

Table C.—Percentages of Physiologically Active Hydrochloric Acid secreted by Normal Mice during Varying Periods of Digestion.

Period of Digestion, half an hour.					
(*a*) Over 12 months.				(*b*) Under 3 months.	
Series 12. Feb. 4, 1908.		Series 16. Feb. 11, 1908.		Series 14. Feb. 7, 1908.	
No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.
6	0·0611	2	0·1243	6	0·2167
1	0·1790	4	0·1476	4	0·1438
1	0·1817	1	0·1765	1	0·1788
1	0·1976			2	0·1962
1	0·2037				
10	0·1129 (av.)	7	0·1451 (av.)	13	0·1881 (av.)

Period of Digestion, one hour.							
(*a*) Over 12 months.		(*b*) Under 3 months.					
Series 6—10.* May 30, 1906—Feb. 28, 1907.		Series 19. March 4, 1908.		Series 20. March 11, 1908.		Series 21. March 17, 1908.	
No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.
32	0·1374	4	0·1353	12	0·1005	14	0·1260
16	0·1992	2	0·1654	4	0·2006	2	0·1397
40	0·1188	11	0·1686	2	0·2845	4	0·1508
30	0·1322						
26	0·1974						
144	0·1488 (av.)	17	0·1604 (av.)	18	0·1432 (av.)	20	0·1323 (av.)

* Repeated here from p. 449 for purposes of comparison.

Table C—*continued.*

Period of Digestion, one hour and a-half.							
(*a*) Over 12 months.				(*b*) Under 3 months.			
Series 11. Feb. 4, 1908.		Series 15. Feb. 11, 1908.		Series 13. Feb. 7, 1908.		Series 18. Feb. 25, 1908.	
No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.
6	0·1515	1	0·1575	6	0·2002	12	0·1716
1	0·2310	4	0·1673	2	0·1894		
1	0·2643	2	0·1684	1	0·1929		
		6	0·2110	4	0·2085		
8	0·1755 (av.)	13	0·1869 (av.)	13	0·2005 (av.)	12	0·1716 (av.)

and 0·1683. All these mice were under three months. The nature of the tumours is indicated in the note to Table D.

Apparently in the experiments relating to mice with transplanted tumours, the greater proportion have attained a maximum at the one-hour period of digestion.

Comparing the general results both for normal mice and for mice with transplanted tumours, it is obvious that there is no general tendency to a decrease in the latter as regards secretion of hydrochloric acid, the variations in both cases being strictly comparable, and, in fact, if we take the average of all mice examined in the two classes, we find that for periods of digestion of one hour and of one hour and a-half, 245 normal mice gave an average of 0·1546 per cent., while 290 mice with transplanted tumours during the same periods of digestion gave an average of 0·1673 per cent., that is a slight *increase*. Again, in the experiments made without reference to time of digestion (Table A), 150 normal mice gave an average of 0·1121, and 178 mice with transplanted tumours gave an average of 0·1752.

Only quite recently we have had the opportunity of examining a series of rat stomachs also supplied by Dr. Bashford, and as these varied in weight (after one hour's digestion) from 2·5 to over 10 grammes, it was a comparatively easy matter, and obviously advantageous, to analyse them singly.

We examined 13 such stomachs after one hour's digestion, three from rats which had been inoculated from tumours identified at the Imperial Cancer Research Laboratories as Flexnor 15B and 15A, and four from rats which had been inoculated from a tumour known as Jensen 8A.

Table D.—Percentage of Physiologically Active Hydrochloric Acid in Mice with Transplanted Tumours.

Period of Digestion, 1 hour.

(*a*) Over 12 months.				(*b*) Under 3 months.			
Series 6—10.* May 30, 1906—February 28, 1907 (non-ulcerated).		Series 6—8. May 30, 1906—January 15, 1907 (ulcerated).		Series 25. May 15, 1908.		Series 27. May 21, 1908.	
No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.
35	0·1393	21	0·2439	4	0·1856	1	0·2102
15	0·1569	11	0·1898			4	0·1421
50	0·1660	20	0·1856			1	0·1642
45	0·1509*					1	0·1553
38	0·1964						
183	0·1627 (av.)	52	0·2100 (av.)	4	0·1856 (av.)	7	0·1569 (av.)

* For tumours in Series 6—10 see notes to Table B. In series 25 the tumour was "No. 27" adeno-carcinoma, and in Series 27 squamous cell carcinoma.

Period of Digestion, 1½ hours.

Mice under 3 months.†

Series 22. May 5, 1908.		Series 23. May 6, 1908.		Series 24. May 9, 1908.	
No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.
6	0·1527	6	0·1290	5	0·1236
6	0·1683	2	0·1151	2	0·1218
		3	0·1272	2	0·1013
				2	0·1530
12	0·1605 (av.)	11	0·1260 (av.)	11	0·1246 (av.)

† In Series 22, the tumour was squamous cell carcinoma and alveolar-carcinoma (Jensen) respectively. In Series 23 they were all squamous cell carcinoma, and in Series 24 the first three were "No. 27" and in the last was alveolar carcinoma (Jensen).

These seven had all developed tumours ranging in weight from 0·3 to 15 grammes. The remaining six had been inoculated with the same tumours, but in all of them the result of such inoculation had been completely negative.

Table E shows a brief summary of the results obtained in both series.

Table E.—Percentages of Physiologically Active Hydrochloric Acid in Stomachs of Rats with Transplanted Tumours (positive), and in Normal Rats (negative); 1 hour's digestion.

Series 26. May 19, 1908.			
No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.
(*a*) Positive.		(*b*) Negative.	
1	0·1143	1	0·1192
1	0·2276	1	0·1076
1	0·2574	1	0·1400
1	0·1835	1	0·1358
1	0·1164	1	0·1564
1	0·2047	1	0·1975
1	0·1822		
7	0·1837 (av.)	6	0·1427 (av.)

It will be seen from the figures given in Table E that for one hour's digestion the rats with transplanted tumours gave an average of 0·1837 per cent. of hydrochloric acid, while those in which result of attempted transplantation had been negative, and which may therefore be regarded as normal, gave an average for the same period of digestion of 0·1427 per cent. The variations from the average are in neither series very considerable.

Finally, we may refer to the examination between May, 1906, and May, 1908, of 15 stomachs of mice affected with tumours which, though undoubtedly cancer, were of entirely spontaneous origin. For these, again, we are indebted to Dr. Bashford.

The following Table F gives a summary of the results obtained.

It will be seen from an inspection of this table that the amount of hydrochloric acid in the gastric contents of mice with spontaneous tumours varied (with one exception, viz., 0·0961 per cent., January 18, 1908) between 0·1372 and 0·2627, with a total average for 15 stomachs of 0·1929 per cent.

Conclusions.—Allowing for individual variations, so unavoidable in physiological experiments, it may fairly be argued that under exactly similar conditions of feeding and of periods of digestion there was evidently no general tendency to decrease in the secretion of hydrochloric acid by mice, either with transplanted or spontaneous tumours (and, so far as our

Table F.—Percentage of Physiologically Active Hydrochloric Acid in the Stomachs of Mice with Spontaneous Tumours.

Date.	No. of stomachs.	Hydrochloric acid.
Period of Digestion, uncertain.		
May 29, 1906.....	1	0·2286
Nov. 4, 1907.....	1	0·1847
Nov. 5, 1907.....	1	0·1372
Nov. 8, 1907..	1	0·1652
Nov. 26, 1907.....	4	0·2136
Jan. 18, 1908.....	1	0·0961
Jan. 18, 1908.....	1	0·1856
Period of Digestion, 1½ hours.		
Feb. 25, 1908.....	1	0·2627
Period of Digestion, 1 hour.		
May 18, 1908.....	1	0·2132
May 18, 1908.....	1	0·1634
May 18, 1908.....	1	0·2120
May 18, 1908.....	1	0·1903
General average		0·1929

experiments go, the same would appear to be the case with rats), but on the contrary, we found a distinct tendency towards increase of the hydrochloric acid.

Consequently it would appear that the conclusion arrived at by Moore and others, that a marked diminution or even an absence of free hydrochloric acid occurs in the gastric contents in malignant disease of organs other than the stomach in human beings, is certainly not borne out by the results of our investigations on the amount of physiologically active hydrochloric acid present in the stomach contents of mice suffering from cancer.

(3) *Experiments made on the Human Subject. (Examination of Test-meals.)*

In the absence of determinations of hydrochloric acid in the gastric contents of man, by the same methods that we had employed in our work on mice, it did not seem possible to arrive at any satisfactory comparison of the results which we have set out in this paper, with those of Moore and his fellow-workers. This being so, it appeared to us to be a matter of importance to carry out a series of estimations on patients suffering from undoubtedly

malignant disease, in situations other than the stomach, and, in addition, to determine the free hydrochloric acid and some other constituents by methods which had also been used by Moore. We therefore commenced in May, 1907, the chemical examination of a series of test-meals from patients suffering from cancer which, by the kindness of Dr. Lazarus-Barlow, of Middlesex Hospital, Dr. Murrell, of Westminster Hospital, and Mr. Leaf, of the Cancer Hospital, we were able to obtain from time to time. Since the above date we have examined 34 test-meals. The results obtained as regards physiologically active hydrochloric acid, inasmuch as they appear to confirm our conclusions with reference to mice stomachs and to contradict or materially modify the conclusions arrived at by Moore and others, will be of interest and, in fact, become a necessary adjunct to this research.

The test-meal given in all cases consisted of one pint of tea and a large round of toast. It was given as usual in the morning, fasting, and was withdrawn one hour after administration. Ewald, with whom the suggestion of test-meals originated, laid down as an essential condition that the test-meal should be withdrawn without the addition of water. Apparently, however, whether owing to difficulty of manipulation (which in some cases is considerable) or from a lack of appreciation of this fundamental rule, water is occasionally added by some operators to aid the withdrawal of the test-meal. We found that in the test-meals supplied to us, as a matter of fact, a number of the earlier ones had been thus diluted, and it was, in fact, due to the much larger quantity of hydrochloric acid found in some later test-meals (which proved on enquiry to have been undiluted), that our attention was especially drawn to this unforeseen contingency.

Thus in Cases 1 to 4 (Middlesex Hospital), the hydrochloric acid found was approximately 0·06 per cent., while in Case 5 (Westminster) we found nearly 0·35 per cent. Again, Case 6 (Middlesex) gave us 0·09 per cent., while Cases 7 to 9 (Cancer Hospital), gave 0·16, 0·22, and 0·18 per cent., respectively. On enquiry in connection with these greatly differing results we ascertained that in Cases 1 to 4 water had been used in order to aid the withdrawal of the test-meal. On the other hand we learnt that in Case 5 and in Cases 7 to 9 the test-meals had been withdrawn without the addition of water.

Owing to repeated assurances that it was not feasible, except in isolated cases, to obtain the original undiluted meal, we agreed to the suggestion in the first instance with one of the house surgeons at the Cancer Hospital, that 10 ounces only of water should be used to aid the withdrawal of the test-meal, and that if the whole of this amount was not required, the remainder should be added to the fluid withdrawn. The withdrawal of test-meals 11 to 23 was

carried out in this manner, and the hydrochloric acid estimated therein was found to vary between 0·036 and 0·1059 per cent.

The only conclusion we could come to from the results of the analyses of test-meals where a known quantity of water had been used in the withdrawal, is that each and all of them represent a quantity of hydrochloric acid which must be considerably less than the actual quantity originally present, but how much less it is impossible to say. We therefore once more discussed the matter with Mr. Leaf, who fully appreciated the point, and we were able by the exertions of Mr. Allen, his house surgeon at the Cancer Hospital, to obtain nine further test-meals which had all been withdrawn without the addition of any water whatsoever. That the difficulty of obtaining undiluted test-meals is not exaggerated is indicated in a statement made to us by Mr. Allen, who says that on an average, where test-meals had been given, two out of three cases had to be abandoned owing to the impossibility of inducing the withdrawal of the fluid from the stomach of the patient.

Taking now the whole of the 34 test-meals from cancerous patients, we found in five of these in which the amount of water used in the withdrawal was unknown, the average amount of hydrochloric acid was 0·0646 per cent., with a minimum of 0·0552, and a maximum of 0·0895; in 16 cases in which the amount of water used in the withdrawal was known, the average amount of hydrochloric acid was 0·0554 per cent., with a minimum of 0·0127 per cent. and a maximum of 0·1059 per cent.

But in the 13 cases where the test-meals were withdrawn without the addition of any water whatever, in two instances only was the hydrochloric acid as low as 0·064 and 0·073 per cent.; in another exceptional instance it was as high as 0·35 per cent. (cancer of liver), while in the remainder it varied between 0·084 and 0·22, seven of these being over 0·16 per cent., with a total average in all 13 cases of 0·1626 per cent., as the following summary (Table G) will show.

As showing the influence of the addition of water in the withdrawal of the test-meal on the results obtained, the following figures will be of interest:—

Case 8 (Cancer Hospital, October 21, 1907), no water used. Found 0·2227 per cent. HCl.

Case 11 (same patient, October 30, 1907), 10 oz. water used. Found 0·0748 per cent. HCl.

Case 24 (Middlesex Hospital, January 28, 1908), 20 oz. water used. Found 0·0127 per cent. HCl.

Case 25 (same patient February 4, 1908), 5 oz. water used. Found 0·0858 per cent. HCl.

Table G.—Analyses of Gastric Contents (undiluted Test-meals) of Patients suffering from Cancer.

Case.	Date of receipt of test-meal.	Hospital.	Sex of patient.	Age.	Nature of disease.	Volume of stomach fluid obtained.	Günsburg test.	Total acidity to phenol-phthalein reckoned as HCl.	Total chlorides.	Inorganic chlorides.	Hydro-chloric acid (physio-logically active).	Hydro-chloric acid free and organic-ally combined. (Mörner Sjöqvist.)	Hydro-chloric acid (free) calculated from velocity of inver-sion of methyl acetate.	Organic acids reckoned as hydro-chloric acid.	Total organic acids reckoned as hydro-chloric acid.
Period of Digestion, 1 hour.															
	1907							per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
5	June 21	Westminster Hospital	F	61	Cancer of bile-duct	52 c.c.	Very marked	0·2737	0·3650	0·0182	**0·3468**	0·3525	0·1940	Nil	0·0036
7	Oct. 21	Cancer Hospital ...	F	62	Carcinoma of breast	21 c.c.	Marked	0·1825	0·2190	0·0547	**0·1643**	—	0·0753	0·0182	0·0182
8	Oct. 21	,, ,, ...	F	63	Carcinoma of breast	80 c.c.	Faint	0·2993	0·2482	0·0255	**0·2227**	0·1240	0·0305	0·0760	—
9	Oct. 22	,, ,, ...	F	40	Advanced carcinoma of cervix	35 c.c.	Nil	0·1642	0·2555	0·0730	**0·1825**	—	0·0074	Nil	0·0182
	1908														
26	March 10	,, ,, ...	F	59	Carcinoma of breast	105 c.c.	Nil	0·0839	0·2208	0·1095	**0·1113**	0·0564	0·0045	Nil	0·0255
27	March 13	,, ,, ...	F	50	Carcinoma of cervix (inoperable)	115 c.c.	0·0693	0·1971	0·2774	0·1149	**0·1625**	0·1566	0·0310	0·0346	0·0328
28	March 18	,, ,, ...	F	50	Carcinoma of cervix	137 c.c.	Nil	0·0474	0·1022	0·0382	**0·0639**	0·0376	0·0022	Nil	0·0073
29	March 27	,, ,, ...	F	34	Carcinoma of uterus (inoperable)	60 c.c.	Faint	0·1095	0·2044	0·0730	**0·1314**	0·1128	0·0072	Nil	0·0182
30	March 31	,, ,, ...	M	51	Cancer of stomach	210 c.c.	Faint	0·1606	0·1752	0·0912	**0·0840**	0·0125	0·0036	0·0766	0·1022
31	April 7	,, ,, ...	F	64	Carcinoma of uterus	112 c.c.	0·0803	0·2117	0·3467	0·1277	**0·2190**	0·2146	0·1076	Nil	0·0328
32	May 15	,, ,, ...	F	40	Carcinoma of uterus (recurrent)	65 c.c.	0·0305	0·1533	0·3212	0·1606	**0·1606**	0·1660	0·0401	Nil	0·0876
33	May 21	,, ,, ...	F	62	Carcinoma of breast	148 c.c.	Nil	0·0620	0·1350	0·0620	**0·0730**	0·0673	0·0061	Nil	0·0182
34	May 28	,, ,, ...	F	62	Carcinoma of breast (recurrent)	140 c.c.	0·0182	0·1825	0·2847	0·0931	**0·1916**	0·1848	0·0201	Nil	0·0328
							Average	0·1636	0·2427	0·0801	**0·1626**	0·1142	0·0407	—	0·0306
Normal Case.															
35	June 2	... ...	M	25	Normal ...	90 c.c.	0·1314	0·1861	0·3175	0·0839	**0·2336**	0·2068	0·1195	Nil	0·1004

As already said, we did not confine ourselves in the analysis of the test-meals to the estimation of those constituents only which we had determined in the case of the mice, viz., the total acidity, total chlorides and the inorganic chlorides (from which determinations we deduced the physiologically active hydrochloric acid and the free organic acids), but we also determined the free hydrochloric acid by the methyl acetate process, the free and organically combined hydrochloric acid by the Mörner Sjöqvist process, and also the total organic acids, free and combined, all of these further determinations having been made by Moore in his analyses of test-meals.* We also applied the Günsburg test in all cases, and where a very marked reaction was obtained we also made the test quantitatively.

We will now briefly discuss the results obtained in these estimations.

We have shown above that in 13 test-meals which had been withdrawn without any addition of water whatever, we obtained an average of 0·1626 per cent. physiologically active hydrochloric acid; five of them being above 0·18 per cent. All of these test-meals were obtained from cases of undoubted cancer. The standard amount of hydrochloric acid in the gastric juice is usually quoted as 0·2 per cent., in healthy individuals, and this has been more or less generally accepted, apparently, without any very definite basis of authority. Moore states in his paper of March, 1905, read before this Society, that in aged individuals the free hydrochloric acid is said to be only slightly over 0·1 per cent., in which case the average we find of physiologically active hydrochloric acid (obviously free at some time during the process of digestion), is well over normal, inasmuch as seven out of the 13 test-meals examined were obtained from individuals between 59 and 64 years of age. As regards our estimations of free hydrochloric acid by the velocity of inversion of methyl acetate, which we carried out in the manner referred to by Moore, we obtained an average of 0·0407 per cent., in the 13 undiluted test-meals from cancer patients, with a variation between 0·0022 per cent. and 0·1940 per cent., all the meals having been withdrawn exactly one hour after administration. Moore, in his first paper already alluded to, quotes 12 cancer cases with an average of 0·0039 per cent. of free hydrochloric acid, but in his second paper (May 9, 1906) in the 'Bio-chemical Journal,' vol. 1, p. 274, he quotes 13 additional cases with an average of 0·0515 per cent. free hydrochloric acid, or about 13 times as much as in the previous instances. It certainly seems curious that in two series of cases of typical carcinoma there should be this remarkable difference, and the fact does not suggest that the determination of free

* Ten cubic centimetres of filtered gastric contents were invariably taken for these estimations.

hydrochloric acid is of any diagnostic value, or, as far as we can see, of any practical importance, and it is a striking fact that in 20 hospital cases (non-malignant), Moore found an average of 0·063 per cent. of free hydrochloric acid, an amount which differs but slightly from the average of his second series of cancer cases (0·051 per cent.). Dr. Palmer, employing the same method, estimated the free hydrochloric acid in test-meals from 14 cancer patients, and found an average of 0·0217 per cent. It may be noted that Palmer lays special stress on the necessity of obtaining the withdrawal of the test-meal without any possibility of dilution with water. Moore makes no reference to this point, but in Case 9 states, that instead of 1 pint of tea, 1 pint of gruel and 2 pints of water were administered and withdrawn one and a-half hours afterwards. It is, perhaps, hardly surprising that, under these circumstances, the free hydrochloric acid obtained was only 0·001 per cent.

It is of importance to note, also, that the interval between the administration of the test-meals and its withdrawal, as recorded by these observers, varied between the wide ranges of one to two hours, and in a single case was as much as three hours. This absence of uniformity as regards time of withdrawal clearly detracts from the diagnostic value of the results, and it is sufficiently obvious that where a number of experiments are being made under varying conditions of time, whether by one or more observers, no proper comparison will be possible between the results or the deductions drawn from them.

We may next discuss the results of the Mörner Sjöqvist estimations. This method is intended to estimate both the free hydrochloric acid and that combined with proteid or organic bases. The underlying principle is that, on bringing the filtered gastric contents into contact with pure barium carbonate, these various forms of hydrochloric acid interact with it to produce barium chloride, and that, on subsequent evaporation, incineration, and extraction with water, the amount of barium chloride formed may be estimated as barium sulphate and expressed in terms of the original hydrochloric acid. In 16 out of 34 test-meals examined, the result obtained by this method agreed almost theoretically with the physiologically active hydrochloric acid found by the method of precipitation with silver; in the remainder it was less, in some cases considerably so. The failure to estimate the full amount of hydrochloric acid in these cases we account for by the presence of mucus-like substances in the fluid examined which, by preventing complete contact of the solid barium carbonate, causes loss of hydrochloric acid during evaporation. The fact, however, that in several cases the method gave results strictly comparable with our silver determinations, entirely disposes of the objection

raised by Moore that the method is liable to estimate other inorganic acids than hydrochloric, which he suggests might be present. He makes, however, no statement as to the particular inorganic acids intended. Inasmuch as phosphate and sulphate of barium are insoluble salts, there only remains nitric acid, the barium salt of which is decomposed on ignition. In our opinion, this Mörner Sjöqvist method is fairly accurate, but that it is not applicable in certain cases is shown above, and as there are other objections to it, not the least of which is the length of time it requires, we do not see any advantage in its employment, since Volhard's method amply suffices. Neither Palmer, nor Moore, in his second paper, make any allusion to the work of Lüttke or Willcox, in which Volhard's method was employed.

With reference to the estimation of the organic acids, free and combined, we have made estimations in all cases by the method used by Moore, and the average result in 12 undiluted test-meals was 0·0331 per cent., with a variation from 0·0036 to 0·1022 per cent. Here, again, we differ from Moore, whose results are invariably low. We obtained, from our own determinations referred to above, the figures for free organic acids by difference, but in all but four cases they were entirely absent.

So much has been written of late with reference to the Günsburg reaction that it is unnecessary to enter into any detail here. It will therefore suffice to say, as regards our own experience, that in only a very few cases in all the test-meals examined, even those which had been diluted, did we fail to get a more or less marked reaction, and in the five cases in which the test was made quantitatively, three of the results approximated to the figure obtained by the methyl acetate process, while two were distinctly higher.

Conclusions.—It would appear, therefore, from our experiments on test-meals from cancer patients, that the physiologically active hydrochloric acid, although appearing in a few cases to be somewhat low, can, in the majority of instances, be regarded as normal, or even above normal, according to the standard of hydrochloric acid accepted as normal. It may be that possibly the free hydrochloric acid in the gastric contents is liable to be diminished in disease, whether malignant or not; but unless the experiments are made under rigid precautions as to avoidance of dilution in withdrawal of the test-meals, and unless the observations made by different observers have reference to exactly the same period of digestion, any comparison of results and any deductions from them must tend to be fallacious. Moreover, as already shown, free hydrochloric acid in the gastric contents may at any moment become combined with proteids and nitrogenous organic bases, and it is obvious that the rate of secretion of hydrochloric acid in the gastric juice, inasmuch as it varies in healthy individuals, may also be expected to vary

under conditions of disease, and if this be so, then another element of uncertainty is introduced into its determination, however rigid the conditions of the experiment may be.

Dr. Bashford has stated* that on the basis of observations made on almost 3000 mice with propagated cancerous tumours during two years, "the conclusion has been arrived at that the presence of a tumour even of greater weight than the mouse itself does not necessarily involve a disturbance of the normal nutrition which could be regarded as comparable to the cachexia frequently associated with malignant new growths in the human subject."

But inasmuch as recent extensive statistics, collected by the Imperial Cancer Research Fund† from various London hospitals, have shown that cachexia is not a constant accompaniment of cancer in man, might we not expect to find the same compensating influence as regards increased or undiminished secretion of hydrochloric acid in the stomachs of human beings affected with this disease?

It is certainly clear from our experiments, so far as they go, that the percentage of physiologically active hydrochloric acid in the gastric contents of mice and rats is, on the whole, slightly greater when they are the subject of cancerous growths as compared with these animals under normal conditions; and although we cannot, of course, from the comparatively few experiments made by us on the human subject, assert that the secretion of hydrochloric acid in cancer is normal (for in a few cases it was decidedly below normal), yet we think that we have sufficiently indicated that the whole question of the amount of hydrochloric acid in human gastric contents in cancer would repay further investigation, and that it is not justifiable, in the absence of more comparable experiments than those at present available, to conclude that it is always greatly diminished in the presence of this disease.

In conclusion, our thanks are due to Mr. S. Bosworth for his able assistance, throughout the whole of this work, in the details of the numerous chemical estimations involved.

* 'Scientific Reports on the Investigations of the Imperial Cancer Research Fund,' No. 2, 1905, p. 40.

† 'Transactions of the Epidemiological Society of London' (N.S.), vol. 26, 1906—7.