Hegsted, D. M., Tsongas, A. G., Abbott, D. B. & Stare, F. J. (1946). *J. Lab. clin. Med.* **31**, 261.
Kibbrick, A. C. & Blonstein, M. (1948). *J. biol. Chem.* **176**, 983.
Kung, L.-C. & Fang, W.-Y. (1935). *Chin. J. Physiol.* **9**, 375.
Kung, L.-C. & Yeh, H.-L. (1938). *Chin. J. Physiol.* **13**, 285.
Macy, I. G. (1942). *Nutrition and Chemical Growth in Childhood*, vol. 1. Springfield, Ill.: C. C. Thomas.
Markham, R. (1942). *Biochem. J.* **36**, 790.
Maroney, J. W. & Johnston, J. A. (1937). *Amer. J. Dis. Child.* **54**, 29.
Mitchell, H. H. & Beadles, J. R. (1937). *J. Nutr.* **14**, 597.
Mitra, K., Verma, S. K. & Ahmed, S. (1948). *Indian J. med. Res.* **36**, 261.
Patwardhan, V. N., Mukundan, R., Rama Sastri, B. V. & Tulpule, P. G. (1949). *Indian J. med. Res.* **37**, 327.
Platt, B. S. (1945). *Brit. med. Bull.* **3**, 179.
Ray, S. N. & Ganguly, R. (1938). *Indian J. med. Res.* **26**, 459.
Read, B. E. & Wong, S. Y. (1923). *Philipp. J. Sci.* **22**, 127.
Wang, C. F. (1936). *Chin. J. Physiol.* **10**, 135.
Wang, C. C., Hawks, J. E. & Hays, B. B. (1928). *Amer. J. Dis. Child.* **35**, 968.
Wang, C. C. & Kaucher, M. (1929). *Amer. J. Dis. Child.* **38**, 468.
Waterlow, J. (1950). *Lancet*, **258**, 908.
Whitacre, J., Willard, A. & Blunt, K. (1929–30). *J. Nutr.* **2**, 187.
White House Conference on Child Health and Protection (1932). *Growth and Development of the Child: III, Nutrition*. New York: Century Co.
Wong, S. Y. (1928). *Chin. J. Physiol. Rep. Ser.* **1**, 123.

The Effect of Fruit-syrup Supplements on the Assimilation of Food from the Alimentary Canal

1. Their Effect on the Digestion of Milk in the Stomachs of Young Rats

BY H. T. FAWNS AND G. H. BOURNE
Departments of Biochemistry and Histology, London Hospital Medical College, Turner Street, London, E. 1

(*Received* 14 *December* 1951)

The increasing consumption in recent years of fruit juices and syrups manufactured from them makes it a matter of some importance to investigate their effect on the digestion and assimilation of other foodstuffs taken concurrently. This applies rather particularly to milk, with which such syrups and juices are often mixed. Here the two matters of chief interest are, first, the effect of fruit juices on the consistency of the clot and its speed of breakdown, and secondly, their effect on the secretion of gastric juice. Fruit juices and their products contain considerable amounts of titratable organic acids, which might be sufficient to alter the pH of the stomach contents and have a significant effect on both these factors.

Earlier investigations appear to have been carried out chiefly on mixtures of milk with fresh orange juice or lemon juice. Thus, Barenberg, Abramson & Messer (1930), in reporting their clinical experience over 5 years, during which 145 infants received a mixture of lemon juice and milk for 6–12 months, claimed that these infants grew better than those receiving milk alone, and that the incidence of diarrhoea was reduced. Woodward (1930) reported similar favourable results with orange juice and milk in infant feeding. One ounce strained orange juice was added to 16 oz. milk.

The curds from these mixtures were very fine and the stools were similar to those of breast-fed infants. Claytor, Smith & Turner (1941), in a study of fifteen subjects, showed that an orange-juice test meal stimulated a high free gastric acidity, which reached its peak in 60 min and remained in the stomach for 105 min.

Mixtures of milk with orange or tomato juice have been given to subjects with uncomplicated duodenal ulcers by Blankenship & Oatway (1931). The orange-juice mixture gave a soft, fine curd and appeared beneficial, whereas the curd with tomato juice was very tough. Matzner & Windwer (1942) report favourably on the treatment of thirty-five consecutive cases of peptic ulcer, both gastric and duodenal, with an orange juice and milk mixture. Since the free acid in the fruit juice would decrease the acid-binding power of the milk, these workers increased the buffering effect of the mixture by the addition of gelatine. No intractable haemorrhages were encountered in their series, and samples of gastric contents removed after administration of the mixture revealed a curd of soft, smooth texture, which they point out is of obvious value in preventing trauma to an organizing blood clot in the crater of a peptic ulcer. Yeagley & Cayer (1948), after treating fifty-one cases of peptic ulcer with fruit juices, emphasize that such juices should only be taken in conjunction with other foods of adequate buffering power in view of their high free acid content. Apart from these considerations, most workers agree that many fruit juices also supply much needed additional ascorbic acid to patients with peptic ulcers and serve to increase the palatability and decrease the monotony of a milk diet. Whereas the work just quoted was carried out in America with fresh lemon or orange juice, in this country commercial fruit syrups made from home-grown varieties of fruit, e.g. black currant, raspberry and strawberry, are commonly used. These are often added as flavouring material to 'milk shakes', both in the home and at milk bars.

EXPERIMENTAL

Plan of experiment

In order to observe the effect of these fruit syrups on the digestion of milk in the stomach, the following investigation was carried out on groups of young rats. Three fruit syrups, admixed with milk, were fed to three groups of rats, while two control groups received milk with an equivalent amount of 50% (w/v) sucrose solution, this being the concentration used in the manufacture of the syrups. There were four rats in each group. The animals were killed by chloroform at 15, 30, 60 and 90 min after feeding and their stomachs excised intact, and opened. The clots were placed in fixative for photography and examination by histological methods. The exuded gastric juice was drawn off separately for estimation of free and total acidity, total and mineral chloride, and of peptic activity where the size of the sample permitted.

Preparation and administration of the syrups

Loganberry, raspberry and strawberry syrups were used, having a high, intermediate and low titratable acidity respectively. The syrups and milk were chilled in the ice-chest before mixing and the syrup was added to the milk from a pipette with constant stirring. By this means a homogeneous mixture was obtained and premature clot formation avoided. The mixture consisted of four parts of milk to one part of syrup (or 50% sucrose in the control groups), this being the ratio commonly employed in making 'milk shakes'.

Natural feeding was considered preferable to administration by stomach tube, since the nervous excitement and possible trauma resulting from passage of the tube might have an adverse effect on gastric secretion. The rats, which were all from the same stock colony and fed on a stock diet, were starved of food and water overnight, usually about 18 h. Next morning the syrup-milk mixtures were placed in the water-bottles. The four rats in a given group would compete eagerly for this bottle, and after about a minute, three other bottles were inserted in the cage, to which the less successful competitors would soon go. By this means it was possible to ensure that within the space of 5–10 min all four rats had filled their stomachs to capacity with the allotted mixture. Experience showed this method to be superior to attempts at individual feeding.

At intervals of 15, 30, 60 and 90 min after feeding, a rat was removed, killed by chloroform and the stomach dissected out.

Collection and examination of the stomach contents

Juice. In most instances, the fundus of the stomach was distended and the outline of the clot, surrounded by a halo of clear juice, could be seen through the wall. A small incision made with fine scissors enabled most of the free gastric juice to escape into the watchglass, while the clot was retained in the stomach. This juice was drawn up with a 1 ml. Record syringe fitted with a wide-bore needle and transferred to a 10 ml. graduated centrifuge tube.

The amount of gastric juice obtained for analysis varied considerably from one animal to another. The maximum was 4·1 ml. and the minimum 0·3 ml. Generally, about 2 ml. or slightly more were obtained from the animals killed at 15 and 30 min, but at 60 and 90 min the available juice became progressively less owing to emptying of the stomach. In these latter samples 0·7–1·0 ml. was the amount usually obtained. A minimum total volume of 1·5 ml. was required for micro-analysis, so that when the volume obtained was less than that, even when the fluid exuded from the clot had been added (see below), the juice was diluted to 1·5 ml. with distilled water in a graduated centrifuge tube, and the volume of this dilution allowed for in calculating the results.

By centrifuging the gastric juice for 10 min at 2500 r.p.m., the solid debris was readily thrown down, giving a clear supernatant fluid, readily handled in a micro-pipette. Occasionally a small fatty clot floated on the surface. Three of the samples of juice were slightly blood-stained as the result of trauma during excision of the stomach. In only one sample (a 90 min specimen) was regurgitated bile visible.

Clots. By extending the incision across the fundus of the stomach, the clot was exposed, and transferred by means of blunt forceps to formalin or Zenker's fixative; the former was used when the entire clot was to be photographed and the latter when histological preparations were to be made. The harder clots (15 and 30 min) could be picked up almost intact, but the softer clots (usually 60 and 90 min) oozed out in a semi-solid mass, and specimens had to be gently 'pushed' into the fixative. If these liquid clots were allowed to remain a few minutes on the watchglass they exuded a further amount of fluid which was drawn up with the Record syringe and added to the initial sample of gastric juice if this was inconveniently small. Clots for histological examination were fixed for 2 days in Zenker's fluid and were then washed with several changes of distilled water for 24 h. They were then dehydrated, cleared and embedded in wax in the conventional way. Sections were cut 10μ in thickness and stained with either haematoxylin or eosin. The eosin preparations proved to be the best for microscopical observation.

Chemical analysis of gastric juice

Free and total acid. Where a sufficient sample was available, 1 ml. gastric juice was diluted with 10 ml. freshly boiled distilled water. Failing this, 0·5 ml. was diluted with 5 ml. Three drops of indicator (a mixture of equal parts of Toepfer's reagent and phenolphthalein) were added and the mixture was titrated with 0·01 N-NaOH from a micro-burette, being stirred throughout by a current of CO_2-free air delivered by a thin tube of polythene. The end-point was determined visually by comparison of the titration tubes with two tubes of standard buffer solution to which three drops of the indicator had also been added, one at pH 3·3 for free acid and the other at pH 8·5 for total acid, the colour comparison being made against a white background. Results are expressed in the usual way as the number of ml. of 0·1 N-alkali required to neutralize 100 ml. juice.

Total and mineral chloride were both determined by the micro-Volhard method described by Patterson (1928), 0·2 ml. juice being required for each estimation. The results are expressed as for free and total acid.

Peptic activity was determined by the method of Mett's tubes. The technique for carrying out the test and preparing the tubes of coagulated egg albumin for digestion was essentially that described by Hawk, Oser & Summerson (1947), who suggest the use of 1 ml. juice diluted with 15 ml. 0·05 N-HCl. Since in the majority of our samples this volume could not be spared, the volume of added HCl was scaled down proportionately so as to keep the ratio of juice to acid constant at 1:15 (usually 0·5 ml. juice to 7·5 ml. acid). In all instances, the volume of digesting fluid was sufficient to cover the egg-albumin tubes completely.

Calculation of peptic index. Two tubes were used for each sample, the column of albumin digested being measured off at each end in mm. This gave four readings, the average of which was taken. In accordance with normal practice, the value for peptic index was taken as the square of this reading multiplied by 16 to allow for the dilution.

Determination of titratable acid in the syrups used

For an interpretation of the results of gastric analysis, the values for titratable organic acids in the syrups themselves were also obtained by diluting 1 ml. syrup with 10 ml. water and titrating with 0·1 N-NaOH to a phenolphthalein end-point. The results quoted in Table 2 are also expressed as ml. 0·1 N-acid/100 ml. syrup to bring them into line with the gastric analysis.

RESULTS

Naked-eye and histological examination of the clots. The consistency of the clot appeared to vary to some extent with the acidity of the juice. Control clots from animals that had fed on milk and sucrose syrup were smooth, dense and rubbery. Clots from the stomachs of animals that had consumed milk mixed with strawberry juice were loose in texture and granular in appearance. Clots from animals fed on loganberry juice (the most acid) showed no cohesion and provided merely a series of isolated large granules (see Pl. 1, 1).

Microscopical examination of sections of the clots from the animals given milk and sugar and milk and strawberry juice confirmed the macroscopical appearance. In the former, one-quarter of the clot was composed of thick, dense strands but the other three-quarters were made up of a fine dense network. The meshes of this network varied between 20μ and 100μ in diameter but most of them were between 30 and 50μ. Such a network would make penetration by digestive juices slow and difficult. Both the thick strands, some of which were 100 μ or more, and the thin ones (20–30μ thick) were made up of densely packed granules with no obvious spaces between (see Pl. 1, 2).

The much looser structure of the clot from animals fed on milk and strawberry juice was confirmed under the microscope. Granules in the strands were much more loosely attached. There was only a vague indication of a network, and such meshes as were present had incomplete walls and their boundaries could not be exactly defined (see Pl. 1, 3).

Chemical analyses of the gastric juices. Results for these are set out in Table 1. The values for organic acids are considered separately in Table 2.

DISCUSSION

Before this investigation was begun, preliminary attempts were made to carry out fractional test meals on rats by means of a small stomach tube. It was hoped by this means to feed both syrup-milk mixtures and sucrose-milk mixtures to the same group of rats in successive weeks, so that the difference in response to each type of test meal could be followed in the same individual animals. Unfortunately, all attempts at withdrawing the stomach contents proved unsuccessful and had to be abandoned. Consequently, the method described had to be adopted and one animal of each group killed for each time interval. Results were, therefore, more liable to be influenced by the variations in response of individual animals than would otherwise have happened, since, under the original scheme, the same rats would have been used for both types of test meal and their idiosyncrasies would have been common to both sets of results. Unfortunately, too, no information is available about the amount and nature of the gastric residuum in fasting rats.

In view of this, it is better when considering these values for gastric analysis to take the mean and the maximum and minimum values for the whole of the experimental groups (receiving fruit-juice syrups) and compare them with the corresponding figures for the whole of the control animals (receiving sucrose syrup).

Table 1. *Analysis of gastric juice. Results for acid and for chloride are expressed as ml. 0·1 N per 100 ml. gastric juice*

Syrup given	Time after feeding (min)	Acid: Free	Acid: Total	Chloride: Total	Chloride: Mineral	Chloride: HCl	Peptic index
		A. Control groups (four rats in each)					
Sucrose solution, 50% (w/v)	15	Nil	21·9	23·3	20·0	3·3	64
		Nil	18·8	40·0	31·6	8·4	25
	30	Nil	19·0	20·0	18·3	1·7	108
		Nil	24·6	40·0	31·6	8·4	32
	60	Nil	28·4	23·5	11·75	11·75	213
		Nil	42·0	60·0	25·0	35·0	12
	90	Nil	56·0	15·2	15·2	Nil	234
		Nil	51·0	48·3	23·0	25·0	55
	Average	Nil	32·7	—	—	11·69	93
		B. Experimental groups (four rats in each)					
Strawberry	15	Nil	23·1	8·3	—	—	—
	30	Nil	23·7	31·6	25·0	6·6	324
	60	Nil	51·8	25·0	15·0	10·0	196
	90	Nil	80·1	45·0	23·3	21·7	135
	Average	Nil	44·7	—	—	12·8	218
Loganberry	15	Nil	62·4	33·3	16·6	16·7	3
	30	Nil	70·6	40·8	20·4	20·4	28
	60	Nil	77·0	81·6	40·0	41·6	24
	90	Nil	62·5	36·6	16·6	20·0	Nil
	Average	Nil	68·1	—	—	24·7	14
Raspberry	15	Nil	46·8	35·0	18·3	16·7	—
	30	Nil	43·8	30·0	25·0	5·0	—
	60	Nil	48·6	41·7	31·6	10·0	—
	90	Nil	56·4	36·6	18·3	18·3	—
	Average	Nil	48·9	—	—	12·5	—

Table 2. *Acid content of gastric juice expressed as ml. 0·1 N-acid/100 ml. gastric juice*

Syrup given	Constituent: Total acid	Hydrochloric acid	Organic acid (by difference)	(A) Excess organic acid over sucrose controls	(B) Titratable organic acid of syrups	Ratio B:A
	A. Control groups (eight rats)					
Sucrose solution, 50% (w/v)	32·7	11·7	21·0	—	—	—
	B. Experimental groups (four rats in each)					
Strawberry	44·7	12·8	31·9	10·9	335	30·7
Loganberry	68·1	24·7	43·4	22·4	530	23·7
Raspberry	48·9	12·5	36·4	15·4	415	26·9

Free and total acidity. No trace of free acid was found in any of the gastric juices examined, the colour on adding indicator being always the yellow of an 'overshot' Toepfer end-point. Therefore, even with a syrup of high titratable acidity and in the presence of maximal secretion of gastric hydrochloric acid, the buffering power of the milk present in these 4:1 mixtures was sufficient to prevent the appearance of enough free acid to give a titratable value; that is to say, the gastric acidity never went below about pH 4.

The values for total acid were considerably higher in the syrup-fed animals than in the controls. The values for organic acids, set out in Table 2, suggest that this increase was due to the organic acids present in the syrups.

Organic acids. In gastric analysis, the value for 'organic' acids is usually obtained by subtracting the value for total hydrochloric acid (free and buffered) from the value for total acid. Table 2 shows the values for organic acid for the three syrup-fed groups calculated on this basis, together with the average value for the control animals given sucrose. If the organic-acid value for the gastric juice of the sucrose controls is subtracted from that in each of the three fruit-syrup groups, the increase in organic acid for each of these groups can be seen. Comparison of these values with the titratable acid of the particular fruit syrup fed, shows them to occur in the same order of magnitude, loganberry being most acid, strawberry least acid and raspberry intermediate between the two (see Table 2).

Hydrochloric acid and peptic acitivity. Values for total hydrochloric acid are usually considered as being the difference between the total and mineral chloride. This includes both free and buffered hydrochloric acid (in this experiment, presumably all buffered). A comparison of the figures for hydrochloric acid and also for peptic activity in Table 1 indicates a trend towards higher values for both these in the fruit-syrup groups. It should be noted, however, that there was considerable batch-to-batch variation in the total and mineral chloride and in the peptic index, so that no exact comparisons are possible nor can any quantitative conclusions be drawn with certainty. To some extent this also applies to the differences for organic acids given in Table 2.

Clots. Although an increase in the secretion of hydrochloric acid and pepsin in the syrup-fed animals has not been conclusively proved, there was an undoubted difference in the nature of the milk clot. Those from the syrup-fed animals were of semi-solid consistency and in a fine state of division, even in the 15 min samples. The sucrose-milk clots tended to be thicker and more cheesy in the 15 and 30 min samples and only showed signs of disintegration after 60 or 90 min. This difference in naked-eye appearance was confirmed by histological examination. The action of these fruit syrups on the milk clot in the stomach would thus appear to be similar to that of the citrus-fruit juices used by the earlier workers cited at the beginning of this paper.

Emptying time of the stomach. With one exception, the stomach was always distended at 15 and 30 min. The exception may have been due to a small intake in that particular instance. In two animals, the stomach was still comparatively full at 60 min and in one at 90 min. Generally, however, it was about half empty at 60 min and rather more than half empty at 90 min. Difficulties often encountered in collecting a sample at

90 min suggested that it would have been unprofitable to continue the experiment beyond that point.

No differences in the rate of emptying of the stomach could be observed between the two groups.

SUMMARY

1. Three fruit syrups mixed with milk in the ratio 1:4 were administered as test meals to groups of rats, and the stomach contents compared with those from control animals receiving milk mixed with an equivalent amount of sucrose.

2. No free acid was observed in any sample of the gastric contents, so that the buffering power of the milk present appears to have been adequate.

3. The total acid was higher in the syrup-fed animals. This can be attributed in some measure to the organic acids present in the fruit syrups.

4. The values for total hydrochloric acid and peptic activity tended to be higher in the syrup-fed animals, but batch-to-batch variations were so large that it is impossible to draw exact conclusions.

5. The milk clots from the stomachs of syrup-fed animals were of finer consistency and disintegrated more rapidly than those from the sucrose controls. Naked-eye appearance was confirmed by histological evidence. This finding agrees with the results obtained on human subjects by earlier workers, who used a mixture of milk and fresh citrus juices.

6. No difference could be observed between the two groups in regard to the emptying time of the stomach.

Our thanks are due to Dr H. E. Magee for his interest and encouragement in this work, to Messrs H. W. Carter and Co. for supplying the syrups and a grant in aid of expenses, and to the Express Dairy Co. Ltd. for a supply of standard milk.

REFERENCES

Barenberg, L. H., Abramson, H. & Messer, W. H. (1930). *Amer. J. Dis. Child.* **39**, 948.

Blankenship, R. C. & Oatway, W. H. (1931). *Ann. intern. Med.* **4**, 1257.

Claytor, F. W., Smith, W. L. & Turner, E. L. (1941). *Amer. J. dig. Dis.* **8**, 43.

Hawk, P. B., Oser, B. L. & Summerson, W. H. (1947). *Practical Physiological Chemistry*, 12 ed. p. 347. London: J. & A. Churchill.

Matzner, M. J. & Windwer, C. (1942). *Rev. Gastroent.* **9**, 105.

Patterson, J. (1928). *Lancet*, **214**, 492.

Woodward, K. G. (1930). *Illinois med. J.* **57**, 210.

Yeagley, J. D. & Cayer, D. (1948). *N.C. med. J.* **9**, 555.

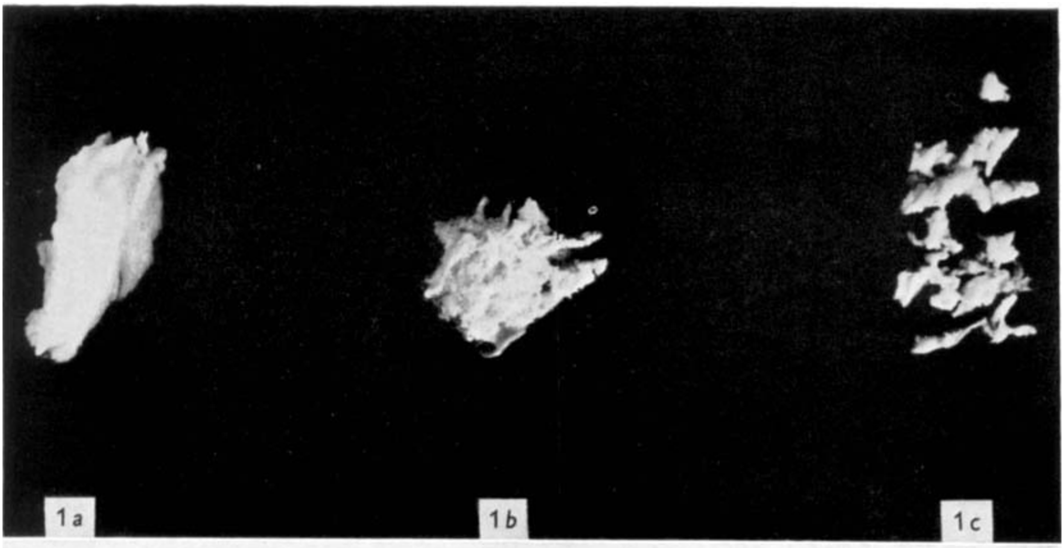
EXPLANATION OF PLATE

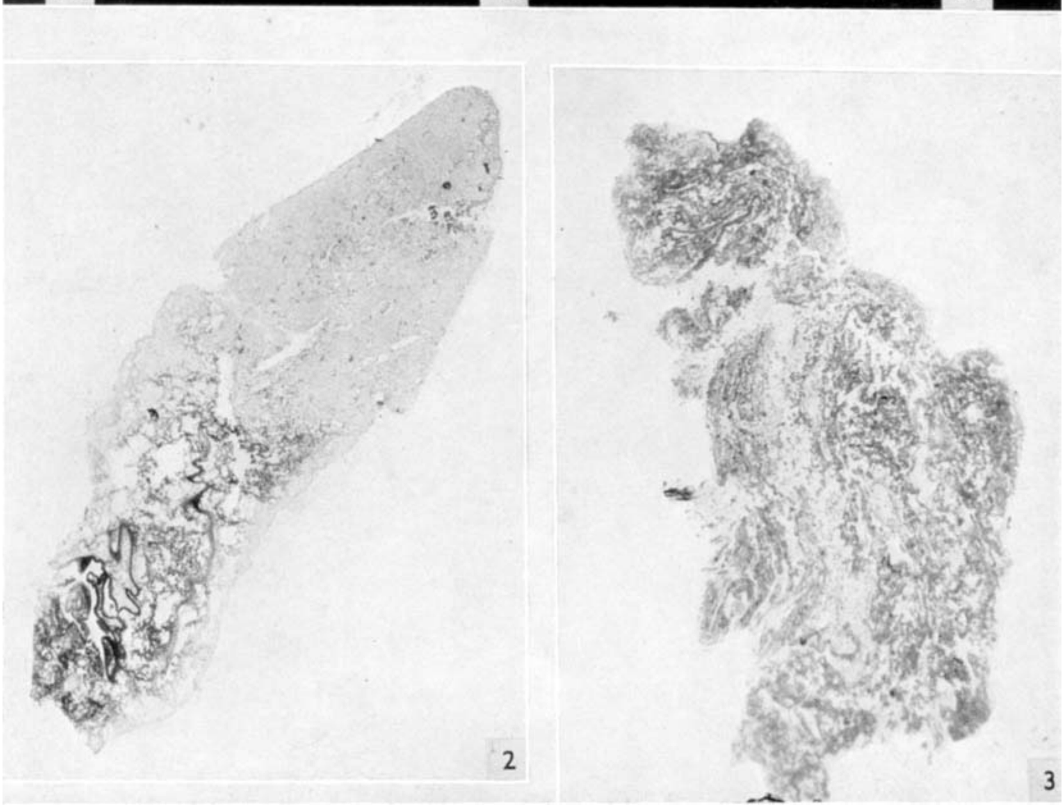
Plate 1

1. (*a*) Macroscopic appearance of milk clot from stomach of a rat fed on milk and sucrose syrup. The dense nature of the clot can be seen. (*b*) Macroscopic appearance of milk clot from stomach of rat fed on milk and raspberry syrup. The loose granular nature of the clot can be seen. (*c*) Disintegrated pieces of clot from stomach of rat fed on milk and loganberry syrup. In this instance no distinct clot was found, only a series of separate granules.

2. Histological section of milk clot from stomach of rat fed on milk and sucrose syrup. Most of the clot is a dense structure difficult to digest.

3. Histological section of milk clot from stomach of rat fed on milk and raspberry syrup. The loose texture which permits relatively easy penetration by digestive juices can be seen.


1a
1b
1c


2
3