work is at present being carried on in the All-India Institute of Hygiene under the guidance of Lieut.-Col. A. D. Stewart, I.M.S. The results of our preliminary observations will be published shortly in the form of a separate paper. The following are among the few observations that have been so far made :—

(*i*) An aqueous extract of bamboo leaves (*Dendrocalamus strictus*) has a definitely lethal action on cyclops.

(*ii*) The aqueous extract and also the expressed juice from young bamboo shoots have a definitely lethal action on guinea-worm embryos, cyclops, and also on the eggs, larvæ and imagines of both mosquitoes and house flies.

(*iii*) The toxic effect of the young bamboo shoot appears to be due to

(*a*) the free hydrocyanic acid formed by the decomposition of one of its cyanoglucosides, as the result of enzyme action and

(*b*) another toxic principle present in the extract, the nature of which is still under investigation.

The practical application of these observations will be discussed in a forthcoming paper. As Leiper has pointed out there still remain for careful study and close observation many interesting problems regarding the prophylaxis of dracontiasis. One can easily realise the limitations of our present knowledge on the prophylaxis of this disease, when it is said that with all the experimental work that has been done on this disease for so many years, the only preventive measure we can advise to the villagers at present is the one enunciated by Manu, *i.e.*, to drink water after straining it through cloth.

I should like to express my grateful thanks to Lieut.-Col. Stewart, I.M.S., for having kindly given me permission to work with him on the larvicidal and other properties of young bamboo shoots, in the All-India Institute of Hygiene. I must also thank Dr. J. V. Karvae, Director of Health in Mysore, for having kindly deputed me to Chitaldrug district for guinea-worm investigation and Drs. A. D. Subba Rao and C. Sree-kantiya for the valuable help given in the course of this work.

Summary

1. For purposes of experimental observation a guinea-worm-infected step-well at Kelgote was treated first with copper sulphate and later twice with perchloron at intervals of 3 days. This measure rendered the well quite free from adult cyclops for a period of about a month. In addition, fish belonging to species *Barbus puckellie* were introduced into this step-well so that these might feed on any cyclops that are likely to be formed from the eggs on which the chemicals used had little or no effect. It is to be seen whether this measure will reduce the number of cyclops in the well permanently and hence also guinea-worm infection in the place. Laboratory experiments show however that this species

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of fish feed voraciously not only on cyclops but also on guinea-worm larvæ.

2. Weak solutions of asafœtida and an aqueous extract of bamboo leaves have a definitely lethal action on cyclops. This property can possibly be made use of in advocating any household prophylactic measures in the guinea-worm infected villages.

3. The aqueous extract and also the juice expressed from the young bamboo shoots have remarkable 'cyclopedocidal', larvicidal, and insecticidal properties.

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A NEW VEGETABLE CULTURE MEDIUM MADE FROM THE PAPAIN DIGEST OF MUNG DAL (*PHASEOLUS MUNGO*), GREEN VARIETY

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THE milky juice of the unripe fruit and also the leaves of the papaya tree (*Carica papaya*, N. O. Passifloreæ) are commonly used by cooks in the East to digest and soften tough meat. The papaya tree was introduced into India by the Portuguese during the seventeenth century. We have not been able to find out the exact date when the green papaya was first used in this country. In 1750 Griffith Hughes in his history of the Barbados wrote 'the juice is of so penetrating a nature that if the unripe fruit when unpeeled is boiled with the toughest old salt beef it will make it soft and tender'. Patrick Brown in 1756 reported upon this remarkable meat-digesting property of the juice of the unripe papaya. The method employed by cooks in India to make the toughest meat tender is first to beat the steak well with an iron pestle or a blunt hatchet, and then to wrap

the meat round with a papaya leaf, or to add a drop or two of the milky juice immediately before cooking. The meat is then placed in a frying-pan with a little fat and fried over an open fire, when it is considered to be grilled by them.

The first chemical investigation of the enzyme action was carried out by Wurtz and Bouchut (1879, 1880 and 1881). Vines (1901) carefully studied the proteolytic action of papain in connection with his extensive study of the proteolytic enzymes of the plant kingdom. He suggested that there were two enzymes, one like pepsin and another like erepsin. Later, it was found that the peptic enzyme had an optimum activity at 80° to 85°C., and was capable of converting the higher proteins into peptones. There was also a second enzyme like erepsin with an optimum activity at 60° to 65°C., which converted peptones into amino-acids, and probably at the same time broke down the higher proteins. Mendel and Blood (1910) studied the action of papain on the vegetable proteins edestin and excelsin, and in 1915 Deleanu studied the hydrolosis of lupine seeds by papain.

In India the digestive ferment papain, present in the unripe papaya fruit and in the juice, should be the enzyme of choice to hydrolyse proteins, when these digested foods are to be given orally. There are religious scruples against using an enzyme derived from animals, such as pepsin from the pig and trypsin from the cow. Papain can digest the higher proteins into amino-acids without any alteration in the pH of the substrate, such as is required for peptic and tryptic digestion. The next difficulty that occurs in India is with Martin's veal broth, which is made from the calf and is objected to on religious grounds by the Hindus. This veal broth is recommended for the manufacture of bacteriophage. To overcome this objection Morison first suggested the use of a papain digest of mutton for the preparation of bacteriophage. Martin (1927) published a short paper on the subject, and later papers on the manufacture of papain-mutton-broth were published by Morison and Vardon (1929) and Morison (1931). The ease with which one can prepare the papain digest of mutton compared with Martin's veal broth has made the former medium a popular one for the preparation of bacteriophage on a large scale in India.

Objections have been raised against the use of meat broth by orthodox Hindus who are strict vegetarians and prefer not to take orally any meat extract if it can possibly be avoided, whilst there are sects in India who prefer their cattle to be killed in an orthodox way by *halal* or *jhutka*. To overcome any possible objection that may be raised by these various sects in India against the use of meat extracts in the preparation of bacteriophage, we decided to investigate the possibility of obtaining the necessary amino-acids from plant proteins by papain digestion. We considered that the amino-acids derived from plant proteins would be more easily assimilated by the bacteria than the amino-acids derived from animal proteins. Moreover, at the same time, these amino-acids derived from plants would be less inhibitory to the multiplication of the bacteriophage. Media containing infusions or digests of plant derivatives of unknown composition have from time to time been used either alone or in combination with additional material of animal origin; thus the Bordet-Gengou medium contains starches and proteins of potatoes in addition to animal proteins. As far as we know no experiments have been recorded with digests of vegetable proteins of more fixed chemical composition such as the lentils or dâls.

Selection of the lentils (or dâls).

The vernacular name *dâl* includes the following dry lentils and peas which are rich in plant proteins, cheap in price, and available in any Indian market. We selected the following varieties of dâls for our experiments:—

1. *Phaseolus mungo* (Mung dâl).
2. *Ervum lens* (Musur dâl).
3. *Cicer arietinum* (Channa dâl), chick pea or gram.
4. *Cajanus indicus* (Arahar dâl).
5. *Pisum sativum* (Matar dâl), dried peas.

The chemical composition of these lentils have been investigated and we give the following table which has been extracted from Stewart, Boyd and Dey's paper (1931):—

TABLE I

Dâl	Protein	Carbohydrate	Fat	Moisture	Ash
Mung ..	25.95	59.88	1.75	8.70	3.72
Musur ..	24.69	63.77	1.08	8.51	1.95
Channa ..	20.57	....	6.35	....	2.15
Arahar ..	22.78	....	1.52	....	3.94
Matar ..	22.28	64.91	1.96	8.17	2.68
Meat* (average)	18.00	....	19.4	60.00	0.96

*Sherman's table as quoted by Hawk (1926).

It will be noted from the above table that the protein content of the lentil—*Phaseolus mungo*—is higher than the protein contents of the other dâls, and a good deal higher than meat. The different dâls were digested under identical conditions. The temperature and duration of the digestion, the relative proportions of the dâls and papain were varied and the different species of broth tested to determine which of the dâls yielded the best broth. This was judged by the freedom from colouring matter, the transparency, and the amino-acid content of the broth. Finally, the growth of the different intestinal micro-organisms were tested upon these different dâl media, using the following media as controls :—

(1) Peptone water, (2) Difco bacto-peptone 1 per cent., and (3) papain digest of mutton containing 0.5 per cent of oxidisable matter. The different media tested showed that the papain digest of mung dâl was by far the best for the growth of intestinal organisms and the preparation of bacteriophage.

The technique for preparing the mung dâl papain broth

Mung dâl according to Watt's *Dictionary of the Economic Products of India* is one of the four pulses which resemble each other very closely in appearance and in habits of growth, the other three being moth (*Phaseolus aconitifolius*), lobia (*Vivna catiang*), and urd or mash (*Phaseolus mungo* variety *radiatus*). There is a certain amount of difficulty in recognising the difference between mung and its subvariety mash. The most popular distinction between these two pulses growing in the field is that mung has dark green leaves and that mash has yellow green leaves, but the chief difference is in the size and shape of the grains. The mash grains are larger and longer than the mung. Mung dâl has three colour varieties; the green seed is the typical and commonest variety, called *hara mung;* the yellow seeds, *Phaseolus aureus,* are known locally as *sona mung—sona* meaning gold; whilst the black seeds *Krishna mung* have been described by Roxburgh as a distinct species under the name of *Phaseolus max.* The mung we used to prepare the broth was *Phaseolus mungo* (the green variety). The reason we chose the green mung dâl was that it is considered to be the most easily digested dâl and is recommended as a food for invalids; it is considered to be 'cooling' and an astringent in its properties. On the other hand *Phaseolus radiatus* (mash) is less digestible but 'heating' and strengthening.

The technique for the preparation of the mung dâl broth, using papain as a digestive ferment, is as follows :—

The composition of the medium

Water—5,000 c.cm.

Mung dâl (powdered green variety)—500 grammes.

Papain (Cawnpore—activity 50 per cent.)—5 grammes.

Sodium chloride in sufficient quantity to make the broth contain a 0.5 per cent. solution of the salt.

The mung dâl is powdered up in a pestle and mortar or more conveniently in a mechanical grinder. Five hundred grammes of this powdered dâl is mixed thoroughly in five litres of water. Five grammes of papain, which we obtained from the Harcourt Butler Institute, Cawnpore, is first made into a thin paste with a little water and added to the mung dâl mixture and then thoroughly mixed. The digestion was carried out in a water-bath at a temperature between 60° to 65°C. for four hours, and during this time the mixture was frequently stirred. We have found that at this temperature the amino-acid digestion is most complete, and the starches are not set free. At the end of four hours the mixture is strained through a fine muslin cloth, and the volume is made up to five litres by the addition of water. The reaction is now adjusted so that a faint alkalinity appears on the addition of ammonium hydrate solution as judged by the appearance of a light red colour, using phenolphthalein as an indicator. The whole medium is now filtered through filter paper. Sterilisation is carried out in the autoclave by heating the broth for 30 minutes at 121.6°C. or 15 pounds pressure. This makes a concentrated broth and also destroys the enzymes. The oxidisable matter is determined by the permanganate method in terms of milligrammes of oxygen required per 100 c.cm. of the concentrated broth. This concentrated broth usually contains oxidisable matter varying between 1.8 to 2.2 per cent. The broth is now diluted with water so as to contain 0.5 per cent. of oxidisable matter. Sodium chloride is added to give a concentration of 0.5 per cent. in the diluted broth. The reaction is now adjusted to a pH of 8.4 which is suitable for most of the intestinal organisms. At the time of adjustment there is a deposit of phosphates. The broth is now filtered through filter paper and should be perfectly clear. The reaction can be readjusted to neutrality if it is required for other types of organism. The broth is distributed into tubes or flasks and sterilised for 20 minutes at 115°C. In the preparation of solid medium a concentrated broth diluted to contain 0.75 per cent. of oxidisable matter is used, and agar added in the usual proportion to solidify the medium.

Notes on the method of preparation of mung dâl medium

The use of caustic soda to adjust the reaction of the broth has the effect of imparting a dark colour to it. Martin (1927) noted this effect of caustic soda in the preparation of mutton broth with papain, especially if an excess of papain has been used in its preparation. Discoloration of the medium, we found, could be

prevented by the use of ammonia. The optimum temperature for the activity of papain varies; at 80° to 85°C., digestion is very rapid occupying about half an hour and the proteins are split into peptones, but there is also considerable difficulty in straining and filtering the digested mass in the case of dâl. At a lower range of temperature, 60° to 65°C. digestion is much slower and it takes four hours to accomplish, but the proteins are converted into amino-acids. At the lower optimum temperature, the starch granules are not broken down, and so do not pass into the broth. The digest is easily filtered so that a very clear broth is obtained for use. The test for the amino-acid digestion is done by using the bromine water test for tryptophane, the reaction becomes positive after two hours digestion and reaches its maximum after four hours. A well-marked cholera-red reaction can be obtained after 24 hours by growing the cholera vibrio in this broth. The broth as prepared above was found to be free from all traces of starches and usually sugar could not be detected, even after the broth had been hydrolysed with hydrochloric acid. Benedict's solution was used for the test to see whether the broth was free from sugar. The amino-acid content of the finished broth, *i.e.*, diluted broth containing 0.5 per cent. oxidisable matter, was found to vary from 3.4 to 4 c.cm. expressed in terms of neutralisation with N/20 NaOH. This compares favourably with the amino-acid content of broth prepared from mutton digested with papain. We also isolated from the mung dâl a more or less pure protein fraction, but we found that the broth made from the proteins was in no way better than the broth prepared from the whole mung dâl. The isolation of protein from the dâl is a very tedious and laborious process so we abandoned the method as it had no advantages over the use of the whole dâl.

Bacterial tests to study the suitability of the mung dâl medium

In India a great deal of the work in bacteriological laboratories is concerned with the growth of the various intestinal organisms. For this reason we selected the following organisms, *i.e.*, cholera and cholera-like vibrios, *B. typhosus*, *B. paratyphosus A* and *B*, *B. dysenteriæ* (Shiga), *B. dysenteriæ* (Flexner) and *B. coli* to test the value of the medium. The medium is very clear and transparent so that we found that it is easy to differentiate the smooth and rough colonies of these organisms. Moreover one could more easily recognise the different colonies seen in mixed growth as obtained from fæces on this medium than in other medium. The bacterial colonies on the mung dâl medium were larger than those on the 0.5 per cent. bile salt McConkey's plate. One has no hesitation in saying that the mung dâl medium is better than the ordinary broth medium for these intestinal organisms.

The next point we studied was the growth of the bacteriophage on the different organisms when grown in the mung dâl medium and in papain mutton broth cultures. Lysis takes place as rapidly and as completely as in the case of the mutton broth medium and the phages were found to be as active when grown on the bacteria in mung dâl medium as compared with those grown on the bacteria in papain mutton broth.

The next test we carried out was the cultivation of ten common fungi obtained from the mycological department of the school. The following fungi were grown on this medium:—

Actinomyces keratolytica, *Trichophyton violaceum*, *Tinea cruris*, *Microsporm auduini*, *Achorion schonleini*, *Actinomyces asteroides*, Monilia from the tongue, *Sporotrichium beurmanni*, and Aspergillus.

Within ten days there was a good growth with all the fungi, in spite of the fact that the pH of the medium was 8.4 instead of 6. The colonies grew well with ærial hyphæ and the deep roots could be seen penetrating the medium when the culture tube was viewed laterally. The medium appears to be extremely useful for the cultivation of these higher fungi. We have now to test the value of the medium for growing delicate organisms belonging to the genera, *Hæmophilus*, *Niesseria*, and other organisms that require the addition of blood to the medium. So far we have found that streptococci of the *fæcalis* type grow well on the medium.

TABLE II

Medium	Raw material used in grammes	Papain used in grammes	Water used in litres	Oxidisable matter. (Approximate).	Volume of the finished broth containing 0.5 per cent. oxidisable matter	Cost per litre
Peptone ..	500	..	50	0.5 per cent.	50 litres	12 annas
Papain—meat ..	500	7.5	2	1.5 „ „	6 „	4 „
Papain—dâl ..	500	5.0	5	1.8 to 2.2 per cent.	20 „	¼ anna

These prices are exclusive of overhead charges.

Finally, when we consider the cost of the medium we find that it is extremely cheap. Thus, for 1 per cent. peptone water, taking the cost of Witte's peptone to be Rs. 28 per pound and Difco to be Rs. 44 per pound, the cost of a litre of peptone water works out at 12 annas to a rupee. With papain mutton broth the cost in India varies between three to five annas per litre, as the cheapest mutton costs about six annas per pound. The mung dâl medium costs a quarter of an anna per litre as mung dâl is only three to four annas per seer, *i.e.*, two pounds. The details of the cost of preparation are shown in table II.

Conclusions

(1) A method is described for preparing a pure vegetable cultural medium, by digesting green mung dâl (*Phaseolus mungo*) with papain at a temperature of 60° to 65°C. for four hours.

(2) The mung dâl broth has given better results in the cultivation of the intestinal bacteria and bacteriophage than mutton broth or peptone water. Solid media made from this dâl broth give a very good growth of the intestinal organisms as well as of the higher fungi.

(3) Mung dâl broth is a pure vegetable medium and is not open to any of the objections that a medium containing meat or one digested by animal enzymes would have, from a religious point of view. In the East this broth can therefore be given orally without offending the religious scruples of the people.

(4) The materials required for the preparation of mung dâl broth are readily obtainable and the cost of production is considerably less than the cost of the other media used in the preparation of bacteriophage.

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CONSTANTS OF PURE BUFFALO *GHI*

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Legal standards in Bengal.—In 1930 we analysed in the Calcutta Public Health Laboratory 572 samples of buffalo *ghi* sent by the local authorities under the Bengal Food Adulteration Act of 1919. According to the standards prescribed by Government under Section 4 of the Act, buffalo *ghi* to be certified as genuine should have the following standards

Butyro-refractometer reading at 40°C., Zeiss scale	.. 40 to 42
Reichert-Wollny value	.. 30
Saponification value	.. 222

Now, out of these 572 samples, 319 or 55 per cent. gave Reichert-Wollny values ranging between 30 and 39 and were of course certified as genuine. But of the remaining 253 samples, while 100 had the values of this constant at 15 or less and so were undoubtedly adulterated,

108 or	18.8	per cent.	gave the	value at	27,
34 or	5.8	,,	,,	,,	,, 24, and
11 or	1.9	,,	,,	,,	,, 21.

In a previous paper (1927) we saw that 8 out of the 51 samples of cow *ghi* prepared by ourselves, *i.e.*, about 16 per cent., had Reichert-Wollny values below 24, and a butyro-refractometer reading at 40°C. from 44 to 45, while the legal standards for these constants of the genuine cow *ghi* were 24 and 40 to 42 respectively; and we remarked that these 16 per cent. of genuine samples of cow *ghi*, each prepared from the milk of a separate cow, would have been condemned as adulterated under the standards of the Act and that such risk, though exceedingly small in the case of market *ghis* which were blends of *ghis* from several cows, could not be ignored. Could the same remarks apply to the empirical standards laid down for buffalo *ghi?*

Genuine buffalo ghi.—With a view to testing the standards for buffalo *ghi*, we prepared the *ghi* ourselves from the milk of individual buffaloes according to the process of the local milkmen, just as we did in the case of the cow *ghi*. We made altogether 51 samples, *viz*, 27 in 1927 and 24 in 1930 and examined them duly after their preparation.

Analysis.—The analysis was done in duplicate and I am much indebted to my assistants Messrs. P. B. Mandal and A. C. Das Gupta for the help I got from them. The values of the

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