TABLE I.
Influence of Aqueous Extracts of Beans, Wheat, and Corn on the Digestion of Casein.

| Extract employed | Casein employed, mg | Protein added in extract, mg | Total protein of substrate, mg | Undigested protein if extract digested alone, mg | Total undigested protein, mg | % of total protein undigested |
|---|---|---|---|---|---|---|
| Casein control | 152 | 0 | 152 | — | 9.36 | 6.1 |
| Navy Bean | 152 | 20.3 | 173 | 13.1 | 151 | 87 |
| Soy Bean | 152 | 34.7 | 187 | 20 | 107 | 57 |
| Wheat | 152 | 5.6 | 158 | 2.1 | 12.7 | 8 |
| Corn | 152 | 1.3 | 154 | 1.3 | 14.4 | 9.4 |

original aqueous extract of navy beans at pH 4 and that of soy beans at pH 4 to 5 for 15 minutes decreases the activities of the inhibiting fraction to about one-fourth of the original values. Smaller degrees of inactivation were observed from pH 1 to 7 with a secondary point of increased inactivation at pH 1.

Preliminary observations indicate that bacterial proteolysis is also retarded by this substance.

In view of the behavior of this material and its heat labile properties it is of particular interest to again note the observations of Osborne and Mendel,[1] Hayward, Steenbock, and Bohstedt[2] and others who have called attention to the low nutritive value of the proteins of raw soy beans as compared with other proteins and the marked improvement in their biological value with cooking or heating. Johns and Finks[3] have made similar observations with the proteins of navy beans. The relations between these findings and those of the present report warrants additional attention. Further studies on the characteristics of this factor are in progress.

*Summary.* Aqueous extracts of soy beans or navy beans contain a fraction which inhibits the *in vitro* digestion of milk casein by trypsin. The fraction can be precipitated with acetone. It is partially heat labile at pH 4. Its presence may account for the low nutritive value of raw soy and navy beans.

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## Specific Agglutination of Murine Erythrocytes by a Pneumonitis Virus in Mice.

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Horsfall and Hahn[1,2] reported the isolation of a pneumonia virus from the lungs of normal Swiss mice. This virus is readily identified by means of neutralization tests with specific anti-sera. It has been isolated from several colonies of Swiss mice by the above authors and by later investigators.

We have recently isolated a virus similar to, if not identical with the mouse pneumonia virus of Horsfall and Hahn. The virus in question was encountered during the course of successive mouse-passages of material derived from a human pneumonic lung. It cannot be determined at the present time whether the virus isolated was present in the original human lung or whether it was derived

1 Horsfall, F. L., and Hahn, R. G., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 684.

2 Horsfall, F. L., and Hahn, R. G., *J. Exp. Med.*, 1940, **71**, 391.

from normal mouse lung by subsequent serial intranasal passage of mouse lung suspensions. The virus has not yet appeared in the course of 17 passages of control mouse-lung suspension obtained from our stock of Swiss mice.

The discovery by Hirst[3,4] and by McClelland and Hare[5] of the capacity of influenza virus to agglutinate red cells of chickens and certain other species introduced a new and important technic in the study of influenza virus. This agglutination phenomenon is specifically inhibited by anti-influenza virus serums. Since this original discovery certain other viruses have been found to exhibit similar activity.[6,7]

We have found that the lungs of mice infected with the virus described above contain an agent capable of agglutinating murine erythrocytes and that this agglutination is inhibited by specific anti-sera. In January, 1944, consolidated lung material was obtained at autopsy from a patient, E.S., in the Presbyterian Hospital. The clinical diagnosis in this case was Addison's disease, complicated by either influenzal pneumonia or primary atypical pneumonia. The pathological diagnosis of the lung condition was primary atypical pneumonia. Consolidated portions of the lungs were received directly from the autopsy table. A considerable amount of bloody material exuded from the cut surfaces of the lung and it was noted that there was a very striking spontaneous agglutination of the red blood cells in this exudate. A suspension was prepared from the lung, and this suspension agglutinated guinea pig red blood cells. It did not agglutinate fowl red cells. The agglutination was not inhibited by anti-influenza A (PR8) or anti-influenza B ferret sera.

A 20% suspension of the human lung was inoculated into the amniotic cavity of 11-day-old chick embryos, and after 3 days' incubation the amniotic fluid was harvested and inoculated intranasally into young Swiss mice. Areas of consolidation were observed in the lungs of these mice when they were killed, 7 days after inoculation. Serial passage was carried out at 7-day intervals. By the 5th mouse-passage a fatal pneumonia was established in mice. This pneumonia is apparently caused by a virus since the lungs of infected mice are usually free from culturable bacteria. Furthermore, mouse lung suspensions have been passed through a Berkefeld V-filter and the bacteria-free filtrate when inoculated intranasally in mice has reproduced the observed pneumonitis. Dr. Horsfall has kindly supplied us with anti-PVM (pneumonia virus of mice) rabbit serum, and it has been found that this serum neutralizes the E.S. virus completely at a serum dilution of 1:125 and partially at a dilution of 1:625. The degree of neutralization compared closely in titer with that of a rabbit anti-serum prepared from the homologous E.S. strain of virus and identified the E.S. virus with PVM. When the E.S. virus from mouse lungs was inoculated into the amniotic cavity of developing chick-embryos, it survived for at least 2 days since the amniotic fluid at this time was infective for mice. On the 11th mouse passage the observation was made that the consolidated lungs of mice dying 4-8 days after inoculation produced marked agglutination of the mouse red blood cells exuding from the cut portion of the lung. This agglutination was very striking in Petri dishes in which portions of lungs were placed, but extracts prepared from suspensions of lung made in the usual manner gave only weak agglutination, or none at all. By freezing the lungs, immediately upon removal, and later heating to 80°C an extract can be prepared which gives good agglutination of murine erythrocytes. Agglutinating extracts used in the tests have been prepared in the following manner:

Portions of lungs are removed and frozen immediately. The lungs are kept at −76°C overnight, then removed, placed in a test tube and heated to 75°C for 10 minutes. They are then ground to a smooth paste and 1 cc of saline for each pair of lungs is added. The suspension is then heated at 80°C for 5 minutes, centrifuged, and the supernatant fluid

[3] Hirst, G. K., *Science*, 1941, **94**, 22.

[4] Hirst, G. K., *J. Exp. Med.*, 1942, **75**, 49.

[5] McClelland, L., and Hare, R., *Canadian Pub. Health J.*, 1941, **32**, 530.

[6] Burnet, F. M., *Aust. J. Exp. Biol. and Med. Sc.*, 1942, **20**, 81.

[7] Nagler, F. P. O., *M. J. Australia*, 1942, **1**, 281.

TABLE I.
Inhibition of Mouse Red Cell Agglutination by Specific Rabbit Anti Serum.

| Serum | Serum dilutions (final) 1/20 | 1/40 | 1/80 | 1/160 | 1/320 | 1/640 | 1/1280 | Agglutination by the serum (control) 1/20 |
|---|---|---|---|---|---|---|---|---|
| Normal Rabbit No. 4/44 | ++ | ++ | ++ | ++ | | | | 0 |
| E. S. Rabbit No. 4/44 | 0 | 0 | 0 | 0 | 0 | + | ++ | 0 |
| Normal R. No. 101 | ++ | ++ | ++ | ++ | | | | 0 |
| PVM R. No. 101 | 0 | 0 | 0 | 0 | + | ++ | ++ | 0 |
| Normal R. No. 102 | ++ | ++ | ++ | ++ | | | | 0 |
| PVM R. No. 102 | 0 | 0 | 0 | + | ++ | ++ | ++ | 0 |
| Normal R. No. 103 | ++ | ++ | ++ | ++ | | | | 0 |
| PVM R. No. 103 | 0 | 0 | 0 | ± | ++ | ++ | ++ | 0 |
| Normal R. No. 5/44 | ++ | ++ | ++ | ++ | | | | 0 |
| Normal Mouse Lung R. No. 5/44 | ++ | ++ | ++ | ++ | ++ | ++ | ++ | 0 |
| Normal R. No. 6/44 | ++ | ++ | ++ | ++ | | | | 0 |
| PR8 R. No. 6/44 | ++ | ++ | ++ | ++ | ++ | ++ | ++ | 0 |
| Normal R No. 7/44 | ++ | ++ | ++ | ++ | | | | 0 |
| Flu. B. R. No. 7/44 | ++ | ++ | ++ | ++ | ++ | ++ | ++ | 0 |

Quantities per tube: 0.25 ml serum dilution.
0.25 ml E.S. lung extract, diluted 1/4.
2 drops 10% suspension of mouse red cells.
Tests read at 90 minutes.
++: marked agglutination.
0: no agglutination.

removed and titrated for its capacity to agglutinate mouse red blood cells. Freshly prepared, washed, suspensions of mouse red blood cells are readily agglutinated by the supernatant fluid. There is no agglutination of rat, cotton-rat, guinea pig, fowl, or human Group O erythrocytes. The hemagglutinating agent is relatively stable. It withstands heating at 80°C for 30 minutes, but is destroyed by heating at 100°C for 5 minutes. Infectivity of the virus for mice is completely destroyed by heating at 56°C for 30 minutes. Agglutination takes place readily at room temperature. Extracts prepared in the above manner usually give good agglutination in dilutions up to 1:32 and show traces of agglutination at 1:512.

Rabbit anti-serum was prepared by the intraperitoneal inoculation of 2 cc of mouse-lung suspensions of the virus. This anti-serum inhibits the agglutination of mouse red cells to a titer of 1:640 whereas control normal serum from the same rabbit fails to prevent agglutination in a dilution of 1:20. Anti-PVM rabbit serum supplied by Dr. Horsfall also inhibits agglutination. Sera to be tested for inhibitory power are inactivated at 56°C and are absorbed with normal murine erythrocytes before use. Inhibition of agglutination closely parallels the virus neutralization titer of such sera as have been tested. An example of serum-inhibition of agglutination is shown in Table I.

To date the lungs of 72 mice dying 4-8 days after inoculation of the E.S. virus have been studied and of these only 3 have failed to show marked agglutination of mouse red cells. In mice killed 7 days after inoculation the agglutinating phenomenon is less regular. However, lungs showing extensive consolidation produce agglutination whereas lungs with small lesions usually fail to do so. Dr. Horsfall kindly supplied us with strain No. 15 of his PVM virus. In the course of a few passages of this strain a similar agglutination of murine erythrocytes in the lungs of approximately 30% of mice dying from the infection or killed 7 days after inoculation has been observed. The agglutination phenomenon with this strain has been irregular and as yet extracts prepared from the lungs of mice inoculated with PVM virus have only low

hemagglutinin titer.

Freshly removed lungs from 30 normal Swiss mice, 20 mice infected with Influenza A and 10 mice infected with Influenza B have failed to cause agglutination of mouse red cells. Lung extracts prepared from normal Swiss mice have also given negative results.

*Summary.* A pneumonitis virus has been isolated during the serial passage of human lung material through Swiss mice. This virus is closely related to the mouse pneumonia virus of Horsfall. The lungs of mice infected with this virus contain an agent capable of agglutinating murine erythrocytes. This agglutination is inhibited by specific anti-sera.

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## Observations on Alloxan Diabetes.

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Since the production of necrosis of the islands of Langerhans by alloxan was first reported by Dunn, Sheehan, and McLetchie,[1] permanent diabetes has been produced in rabbits by Bailey and Bailey,[2] in rats by Dunn and McLetchie,[3] and in dogs by Goldner and Gomori.[4] The literature on this subject has been summarized by Joslin[5] and by Bailey, Bailey, and Leech.[6] From our observations confirming the production of alloxan diabetes, we have tried to select those which supplement the data in the literature.

*Methods.* Normal male rabbits were used and were permitted to eat up to the time of injection. A 2 or 5% aqueous solution of alloxan was injected into the ear vein either in a single dose or in 2 or 3 divided doses 15 to 30 minutes apart. Other routes of administration were tried unsuccessfully. During the next 12 hours some of the animals were given varying quantities of 50% glucose by vein as recommended by Bailey and Bailey.[2] Others were furnished an ample supply of food. In most cases these rabbits ate enough to combat the hypoglycemic stage of alloxan poisoning first observed by Jacobs.[7] Hyperglycemia and glycosuria were noted on the following day and generally reached their greatest severity by the 3rd or 4th day after injection. Once established, the diabetes has persisted throughout the period of observation (maximum 9 months).

All animals were kept in metabolism cages and daily urine collections were made, thymol being used as a preservative. The diet consisted of weighed amounts of cabbage, carrots, and oats. The available carbohydrate of the diet was calculated from standard tables, but no attempt was made to estimate the calories available from cellulose. According to Lusk[8] 22% of cellulose may be digested by the rabbit. However, the figures given for fiber content of cabbage and carrots is 1% so that there would be a relatively small correction, approximately 2% of the usual value for available carbohydrate.

Others have observed that effective doses of alloxan were generally fatal to animals under barbiturate anesthesia. The few dogs,

1 Dunn, J. S., Sheehan, H. L., and McLetchie, N. G. B., *Lancet*, 1943, **1**, 484.

2 Bailey, C. C., and Bailey, O. T., *J. A. M. A.*, 1943, **122**, 1165.

3 Dunn, J. S., and McLetchie, N. G. B., *Lancet*, 1943, **2**, 384.

4 Goldner, M. G., and Gomori, G., *Endocrinology*, 1943, **33**, 297.

5 Joslin, E. P., *New Eng. J. Med.*, 1944, **230**, 425.

6 Bailey, C. C., Bailey, O. T., and Leech, R. S., *New Eng. J. Med.*, 1944, **230**, 533.

7 Jacobs, H. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 407.

8 Lusk, G., *Science of Nutrition*, W. B. Saunders Co., Philadelphia, 1931.