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## Recent Deaths

**Russell I. Baker** (59), chemist, Linden, N. J., Dec. 11; **Thomas H. Bissonnette** (66), biologist, Hartford, Conn., Nov. 30; **Beatrice H. Brickett** (87), physician, Newton, Mass., Dec. 10; **Peter H. Buck** ("Te Rangi Hiroa") (71), anthropologist, Honolulu, Dec. 1; **Arthur E. Davis** (74), cancer specialist, Pittsford, N. Y., Dec 7; **Samuel H. Derickson** (—), biologist, Reading, Pa., Nov. 27; **George E. Doke** (74), mechanical engineer, Yonkers, N. Y., Dec. 6; **James E. A. Eggleson** (72), chemical engineer, Essex Fells, N. J., Dec. 14; **Benjamin K. Fletcher** (83), pediatrician, Philadelphia, Dec. 17; **Edmond Fleutiaux** (94), coleopterist, Nogent-sur-Marne, France, Nov. 25; **W. Wallace Fritz** (81), neuropathologist, Lansdale, Pa., Nov. 24; **Edward T. Grandlienard** (72), civil engineer, Drexel Hill, Pa., Nov 27; **Edgar A. Groves** (69), civil engineer, New York, Nov. 25; **Gene W. Hall** (53), civil engineer, Rockville Centre, N. Y., Nov. 29; **William L. Higgins** (84), physician and civic leader, Norwich, Conn., Nov. 19; **Kirke K. Hoagg** (62), engineer, Scarsdale, N. Y., Dec. 11.

**Max Immanuel** (61), economist, New York, Dec. 9; **Robert B. Irwin** (68), former executive director, American Foundation for the Blind, Bremerton, Wash., Dec. 12; **Herbert Jackson** (68), botanist, Toronto, Dec. 14; **Carl O. Lampland** (78), astronomer, Flagstaff, Ariz., Dec. 14; **Walter B. Lancaster** (88), ophthalmologist, Boston, Mass., Dec. 9; **John I. Lauritzen** (67), plant physiologist, Riverside, Calif., Nov. 27; **S. G. Law** (49), neuropsychiatrist, Minneapolis, Sept. 3; **N. J. Lennes** (77), mathematician, Missoula, Mont., Nov. 21; **Samuel Levine** (69), internist, Brooklyn, N. Y., Nov. 29; **Andrew F. Lippi** (71), chemist, Philadelphia, Dec. 13; **David McCoach, Jr.** (64), civil engineer, Washington, D. C., Dec. 15; **Carl J. Moroney** (65), engineer and sugar official, San Mateo, Calif., Nov. 27; **Paul H. Musser** (59), educator, Philadelphia, Nov. 21; **Charles F. Noll** (73), agronomist, State College, Pa., Dec. 18.

**Charles D. Parfitt** (80), of Toronto, tuberculosis specialist, Brookline, Mass., Nov. 20; **Todd M. Pettigrew** (64), geophysicist, Washington, D. C., Dec. 13; **Cesar U. Piedrahita** (64), expert on tropical medicine, Bogota, Colombia, Dec. 17; **Henry S. Raper** (69), physician, Manchester, Eng., Dec. 12; **Jerohn J. Savitz** (85), educator, Westfield, N. J., Dec. 5; **Marc Schuman** (—), inventor, Philadelphia, Nov. 22; **Lemuel F. Smith** (78), chemist, Richmond, Va., Nov. 24; **Angelo L. Soresi** (69), surgeon, Brooklyn, N. Y., Dec. 11; **Leo Spiegel** (72), dermatologist, New York, Dec. 17; **John W. Thomas** (71), chemist and rubber company official, Akron, Ohio, Nov. 26; **Benjamin F. Tillson** (67), mining engineer, Montclair, N. J., Dec. 4; **Theodore P. Walker** (69), industrialist, New York, Nov. 29; **Carl N. Webb** (51), chemist, Oxford, Ohio, Dec. 14; **Archer E. Young** (78), mathematician and research engineer, Mamaroneck, N. Y., Nov. 28.

# *Technical Papers*

## The Site of Nitrogen Absorption in Rats Fed Raw and Heat-treated Soybean Meals

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It is well known that moderate heat treatment improves the nutritive quality of the proteins of soybeans. Several heat-labile fractions which inhibit tryptic hydrolysis *in vitro* have been obtained from raw soybeans. Some of these have been shown to retard the growth of chicks, rats, and mice. This work has been comprehensively reviewed by Liener (*1*), Laskowski (*2*), Almquist (*3*), and Griswold (*4*).

We have studied the effect of heating on the digestibility and growth-promoting value of the proteins of soybean meal for the rat. Our results, like those of other investigators (*5–7*), indicate that properly heated meal has only slightly higher digestibility than raw meal and that this digestibility difference is so small it can hardly account for the observed marked difference in growth-promoting value. This was true when digestibility estimations were based on fecal nitrogen excretion. When contents of progressive segments of intestine were studied, it became apparent that with properly heated meal, all, or nearly all, of the nitrogen that is ultimately to be absorbed is absorbed during passage of the meal through the small intestine. However, with the raw meal, much of the nitrogen appears to be absorbed from the large intestine.

Digestibility estimations were made by the chromic oxide indicator method described by Schürch *et al.* (*8*). Briefly, the method involves incorporation of a known amount of a completely indigestible material ($Cr_2O_3$) in the feed, determination of its concentration in the feces, and determination of the nitrogen content of the feed and of the feces. A coefficient of digestibility for the nitrogen of the feed may then

[1] With the technical assistance of P. R. Stout in statistical interpretation.

TABLE 1

COEFFICIENTS OF DIGESTIBILITY OF SOYBEAN NITROGEN

| Estimated from analysis of feces | | | Estimated from analysis of contents of terminal 20% of small intestine | | | |
|---|---|---|---|---|---|---|
| Ration No. | 564 | 565 | 564 | | | 565 |
| Sample fed | Raw | Heated | Raw | | | Heated |
| Rat No. | *Period 1* | *Period 2* | Rat No. | | Rat No. | |
| 1 | 77.7 | 82.1 | 14 | 61.5 | 28 | 81.4 |
| | 77.1 | 80.3 | 13 | 51.5 | 40 | 81.4 |
| | 77.6 | 81.9 | 27 | 49.1 | 29 | 81.0 |
| | | | 12 | 45.7 | 35 | 79.2 |
| | *Period 2* | *Period 1* | 11 | 44.3 | 22 | 77.5 |
| 2 | 77.4 | 81.2 | 39 | 42.1 | 34 | 77.2 |
| | 75.7 | 80.7 | 32 | 38.5 | 10 | 76.9 |
| | 78.7 | 82.0 | 38 | 37.7 | 23 | 74.7 |
| | | | 26 | 31.5 | | |
| | *Period 1* | *Period 2* | 16 | 29.8 | | |
| 3 | 74.5 | 84.1 | 17 | 28.7 | | |
| | 75.5 | 81.8 | 19 | 25.8 | | |
| | 78.4 | 81.2 | 20 | 24.1 | | |
| | | | 15 | 23.0 | | |
| | | | 33 | −3.5 | | |
| | | | 18 | −7.4 | | |
| Mean | 76.96 ± 1.23* | 81.70 ± 1.23 | Mean | 32.65 ± 13.53 | | 78.66 ± 3.08 |

| *Coefficient of digestibility* | *Mean difference* | *Value of* t | *Probability* |
|---|---|---|---|
| From feces | 4.7 | 7.95 | < 0.0001 |
| From intestinal contents | 46.0 | 9.83 | < 0.0001 |

* Each fiducial limit at 0.01 level.

be calculated from these data through the use of the two nitrogen to $Cr_2O_3$ ratios.

Raw full-fat soybeans were steamed and rolled, and hexane was extracted with minimum heating. Portions of the extracted flakes were heat-treated by autoclaving. A series of 12 samples, representing varying degrees of heat treatment from raw to badly damaged, was prepared. Each sample was fed to weanling rats for determination of the growth-promoting value of its proteins. The growth test data will be reported in detail elsewhere. Two of the samples were used in the digestibility studies reported here: the first was the unheated solvent-extracted flakes and will be termed "raw;" the second gave a near-maximum growth-promoting value (with respect to the 12-sample series) for rats in the above growth tests and will be termed "heated." The treatment given this heated sample was 15 min autoclaving at 121° C. The 21-day mean nitrogen efficiency values (g gain in body wt/g nitrogen intake) obtained with *ad lib.* feeding of raw sample ration and heated sample ration were 6.2 ± 2.0[2], and 13.7 ± 2.0, respectively.

The rations fed in the growth and the digestibility studies were identical in their levels of salt mixture, added synthetic B complex vitamins (including choline chloride, 200 mg/100 g), vitamins A and D, and α-tocopherol. The rations fed in growth tests contained ground soybean flakes to supply 1.6% total nitrogen and corn oil to give a total fat level of 10%; the rations fed in digestibility studies contained soybean meal to supply approximately 3.5% total nitrogen, 5% total fat, and 2.0% $Cr_2O_3$. The remainder of each ration was cornstarch.

Three adult rats were used in the gross digestibility studies. Each was carried through two feeding periods: he was fed raw sample ration during one of the periods and heated sample ration during the other. During each of the two feeding periods, each rat received his respective test ration for at least 2 days before any feces were collected. Three 24-hr collections of feces were made for each rat during each period. Feces were collected on screen in order to minimize contamination with urinary nitrogen. Feces samples were air-dried at room temperature sufficiently for grinding in a mortar. After removal of most of the hair by sieving, the samples were assayed in duplicate for $Cr_2O_3$ and for nitrogen.

The determination of $Cr_2O_3$ was carried out as described by Schürch (*8*) except for minor changes, which were made to increase the sensitivity of the method and to allow the use of smaller samples. Semimicro determinations of nitrogen in feces and intestinal contents were made by the method of Henwood and Garey (*9*), using duplicate 20-mg samples of dry material.

The results of the digestibility studies based on fecal excretion of nitrogen are given in the first section of Table 1. The coefficients of digestibility of 77 for the raw and 82 for the heated meal ration are similar to those reported by Melnick *et al.* (*6*) for raw and properly heated meals fed to rats.

[2] Fiducial limits at 0.01 level.

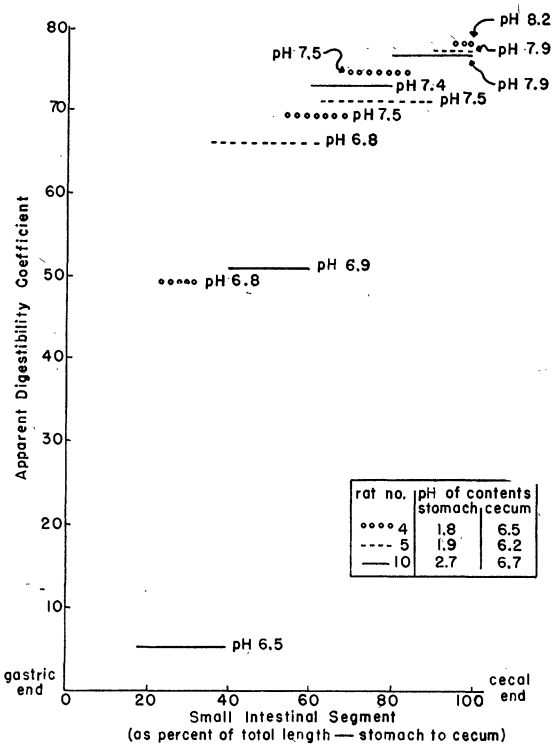


FIG. 1. pH and apparent nitrogen absorption in the small intestine of rats fed heated soybean meal.

The procedure for the study of rates of absorption of soybean nitrogen from the small intestine was as follows. Adult rats were fasted for periods of 24–36 hr. When several such fasted animals were killed (chloroform), the digestive tract was found to be empty except for cecal contents. After fasting, animals were fed the test rations for a period of at least 7 hr. They were then killed, and the digestive tract from esophagus to rectum was removed, freed from mesenteric attachments, and rinsed in cold tap water to remove blood. The small intestine was measured from stomach to cecum and cut into segments of known length and position. These segments were slit open, and the contents carefully removed from the mucosa. Animals were killed individually, and the above operations were carried out as rapidly as possible.

The contents of intestinal segments were placed on watch glasses, and their pH was determined, using a glass-calomel electrode system. They were then placed in a vacuum desiccator to be dried sufficiently for grinding. This required about 3 hr with a high vacuum pump. The dried samples were finely ground in an agate mortar to allow uniform sampling for $Cr_2O_3$ and nitrogen determinations.

The results of preliminary tests on rats fed the heated soybean ration indicated that with these animals near-maximum coefficients of digestibility for nitrogen were obtained with the contents of any segment of the terminal 40% of small intestine (Fig. 1). Contents of successive segments of small intestine of rats fed raw meal showed, in general, a progressive rise from an initial negative value but much lower apparent digestibility coefficients[3] throughout and greater variability.

These preliminary tests indicated that the use of only the terminal 20% of the small intestine would be satisfactory for a comparison of the two soybean rations. Only this segment was opened when the two rations were compared using larger numbers of animals. The same feeding technique and experimental procedure outlined above were employed. Results of these tests are given in the second section of Table 1.

These data indicate that, in rats fed raw meal ration, much of the nitrogen that escapes absorption in the small intestine must later be absorbed from the cecum or colon, since the coefficient of gross digestibility for the nitrogen of this ration is much larger than that estimated from the contents of terminal small intestinal segments. The greater variability of observed small intestinal coefficients of digestibility for animals fed raw meal ration is striking. An analysis of variance showed this difference between raw and heated meal rations to be highly significant.

A comparison of rat growth data with digestibility data suggests that the coefficient of digestibility estimated from the contents of the terminal portion of the small intestine may be closely related to utilization of nitrogen for growth. The grams gain in body weight per gram of nitrogen intake were 6.2 and 13.7 for the raw and the heated meal rations, respectively. Grams gain in body weight per gram of nitrogen absorbed from the small intestine[4] are 19 and 17 for the raw and the heated samples, respectively. Apparently the amino acids (or their nitrogen-containing derivatives) absorbed from the large intestine have little utility for growth, presumably as a result of bacterial activity.

The pH values for intestinal contents of rats of the two groups were similar. The mean values (terminal 20% of small intestine) were $7.82 \pm 0.08$[5] for rats fed raw meal ration, and $7.88 \pm 0.09$ for rats fed heated meal ration. The pH values of cecum contents were more variable and also were not significantly different for animals of the two groups. Mean values were $6.2 \pm 0.4$ and $6.5 \pm 0.6$ for animals fed raw and heated samples, respectively. It seems unlikely that significant "tryptic" hydrolysis could continue in the cecum under such conditions of pH, even though proteolytic enzymes may enter from the small intestine with escaping raw meal protein.

It seems possible that when raw meal protein escapes hydrolysis in the small intestine, substantial quantities of pancreatic or intestinal glandular secre-

[3] These coefficients of "digestibility" might better be termed coefficients of "net absorption," since in reality they represent the net absorption of nitrogen from the alimentary tract, rather than the total absorption of food nitrogen, as percentage of the nitrogen intake. Thus a negative coefficient is observed when nitrogen secretion overbalances nitrogen absorption.

[4] These calculations are based on the assumption that our digestibility data, obtained with adult animals, are applicable to the young animals used for growth tests.

[5] Fiducial limits at 0.05 level.

tions may be lost with it into the large intestine. This is suggested by our observation of several "negative" coefficients. Kunitz (*10*) has reported a stoichiometric, undissociable compound formed by combination of trypsin-inhibitor with trypsin. If significant amounts of secreted proteins are lost in this way, the "methionine-rich" complex observed by Bouthilet *et al.* (*11*) in the feces of chicks fed raw meal could be of endogenous origin. If such lost endogenous protein is high in methionine (relative to soybean protein), the degree of methionine deficiency of animals fed raw meal would be more severe than that estimated by consideration of the amino acid composition of soybeans. Such a sustained loss of secretions may be related to the pancreatic hypertrophy and increased concentrations of trypsinogen and chymotrypsinogen in pancreatic tissue reported in animals fed raw soybeans (*12*).

### References


1. LIENER, I. E. *Trans. Am. Assoc. Cereal Chemists,* **8**, 162 (1950).
2. LASKOWSKI, M. *Ann. Rev. Biochem.,* **19**, 21 (1950).
3. ALMQUIST, H. J. In D. M. Greenberg (Ed.), *Amino Acids and Proteins.* Springfield, Ill.: Thomas, 586 (1950).
4. GRISWOLD, R. M. *J. Am. Dietet. Assoc.,* **27**, 85 (1951).
5. HAYWARD, J. W., STEENBOCK, H., and BOHSTEDT, G. *J. Nutrition,* **11**, 219 (1936).
6. MELNICK, D., OSER, B. L., and WEISS, S. *Science,* **103**, 326 (1946).
7. MITCHELL, H. H., HAMILTON, T. S., and BEADLES, J. R. *J. Nutrition,* **29**, 13 (1945).
8. SCHÜRCH, A. F., LLOYD, L. E., and CRAMPTON, E. W. *Ibid.,* **41**, 629 (1950).
9. HENWOOD, A., and GAREY, R. M. *J. Franklin Inst.,* **221**, 531 (1936).
10. KUNITZ, M. *J. Gen. Physiol.,* **30**, 291 (1947).
11. BOUTHILET, R. J., *et al. Poultry Sci.,* **29**, 837 (1950).
12. CHERNICK, S. S., LEPKOVSKY, S., and CHAIKOFF, I. L. *Am. J. Physiol.,* **155**, 33 (1948).


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## Tissue Hemolysins


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Among the many reports on tissue hemolysin published during the past fifty years, cis-vaccenic acid (*1, 2*) has been the only nonspecific hemolysin isolated from tissue extracts and clearly identified. However, the conclusion by Laser (*3*) that there seems to be no need for further speculation regarding their nature appears hardly acceptable in view of the evidence for the hemolysins found in incubated tissue. Laser's procedure for the extraction of the unsaturated acid from horse brain did not clearly indicate any incubation period or definite opportunity for enzymatic activity, thus suggesting the presence of free cis-vaccenic acid or an unstable complex easily broken by the chemical procedure.

If attention is focused on pathological states, it seems relatively unimportant whether hemolysins exist free in normal healthy tissue or whether they are potentially present as enzyme-substrate-inhibitor systems, capable of producing active hemolysin when disturbed by tissue injury or disease. This has led many previous workers to examine diseased tissue, when available, or more often normal tissue, minced, extracted, and incubated sufficiently to produce substantial amounts of active hemolysin. Such treatment has frequently demonstrated (*4–8*) (*a*) that the hemolysins do not arise from contaminating microorganisms; (*b*) that heat, prior to incubation, prevented the development of hemolysins in tissue extracts; (*c*) that a few hours of incubation greatly increased the hemolytic activity; and (*d*) that the active lysin could usually be extracted with alcohol or ether.

TABLE 1

HEMOLYTIC ACTIVITY OF TISSUE HEMOLYSIN AND OLEIC ACID

| Mg fatty acid* | Time of complete hemolysis (min) | |
|---|---|---|
| | Tissue hemolysin | Oleic acid |
| 0.22 | 1.5 | 3.0 |
| .18 | 3.0 | 4.5 |
| .09 | 12.5 | 40.0 |
| 0.07 | 51.0 | > 90.0 |

* Fatty acid dissolved in 8 ml phosphate buffer, pH 7.3, 2 ml 2% suspension sheep erythrocyte added to each tube.

Experiments in this laboratory have shown that if storage or incubation (4°–37° C), hot solvents, and strong alkaline or acid reagents were avoided, fresh liver, kidney, and testes of rabbit, guinea pig, rat, and cattle yielded no more than traces of hemolytic materials. On the other hand, after a few hours' incubation at 37° C, a strongly hemolytic material could be extracted with cold alcohol.

Current experiments have led to the isolation of a very active hemolysin from incubated saline extracts of bovine testicular tissue. This purified material proved to be a highly unsaturated acid not identical with the octadec-11-enoic acid identified by Morton *et al.* (*2*) in horse brain extracts. It is present in no more than traces in normal healthy tissue, but is readily obtained in the following manner.

Bovine testes were ground, extracted with 0.9% saline, and centrifuged. The supernatant material was titrated to pH 4.5 with acetic acid and centrifuged. The precipitate was suspended in saline at pH 6.2, incubated 8–12 hr at room temperature (20° C), and extracted with ethanol. The active hemolysin in the ethanol extracts was separated from inactive lipoid materials by an extensive series of transfers (detailed elsewhere) between organic solvents and aqueous solutions, at acid, neutral, and alkaline pH. A short bath, low temperature, and low pressure distillation finally yielded a light yellow oil with a molecular weight of 310 + 10 and an iodine number of 257 (Hanus).

Table 1 shows the action of the tissue hemolytic acid in comparison with oleic acid. Hemolysis was inhibited by lecithin but not by cholesterol.

When the precipitate obtained at pH 4.5 was heated at 100° C prior to incubation, no hemolytic