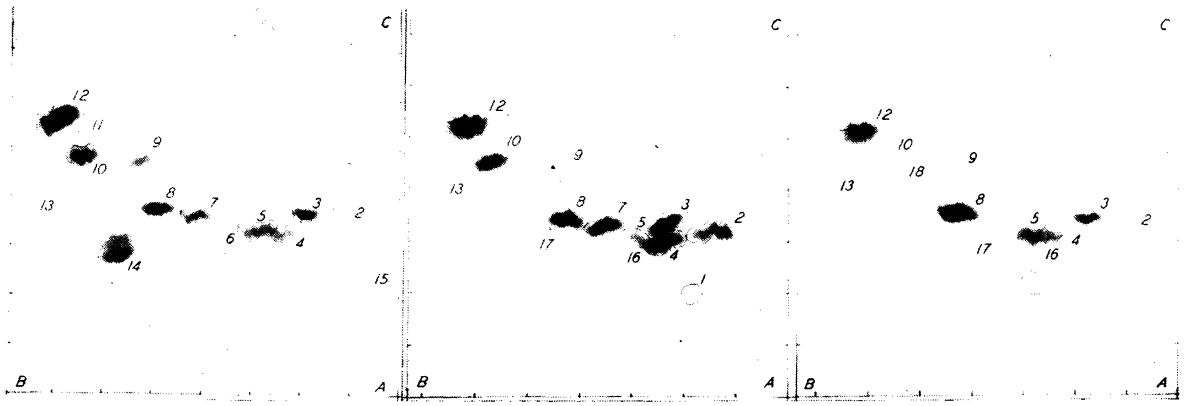

FIG. 1. Paper partition chromatograms of hydrolyzates of tobacco mosaic virus protein; left, pancreatic digest; middle, acid hydrolyzate; right, alkaline hydrolyzate. A–B, water-saturated phenol. A–C, *n*-Butanol-acetic acid-water. Spots: 1, cystine; 2, aspartic acid; 3, glutamic acid; 4, serine; 5, glycine; 6, asparagine; 7, threonine; 8, alanine; 9, tyrosine; 10, valine; 11, tryptophane; 12, leucine, isoleucine, and phenylalanine; 13, proline; 14, a peptide; 15, probably residual protein; 16, lysine; 17, arginine; 18, α-amino-*n*-butyric acid, believed to be an artifact probably originating from threonine.

glass tubes. These were autoclaved for 6 hr at 15 lb pressure. Excess HCl was eliminated by evaporation, and the $Ba(OH)_2$ by precipitation with H_2SO_4.

Each sample was chromatographed routinely in three solvent pairs. As the first solvent, water-saturated phenol was used invariably for all two-dimensional papers. For the run in the second direction, two solvents were employed, either 2–4 lutidine (1 vol) thoroughly shaken in water (1 vol), or freshly prepared *n*-butanol-acetic acid-water. Spots were revealed by spraying the dried sheets with 0.1% ninhydrin in 95% ethanol.

A chromatogram of the pancreatin digest is shown in Fig. 1 (left); the control gave only one faint spot in the position normally occupied by alanine. Asparagine, spot 6, was first tentatively identified by position and by its characteristic rusty color with ninhydrin. Positive identification was later achieved by demonstrating that the asparagine spot was intensified on both phenol/lutidine and phenol/butanol-acetic acid chromatograms by addition of authentic asparagine to the spot at the origin.

It was further shown that acid hydrolysis of the eluate from a cutout of the suspected asparagine spot resulted in its disappearance, with concomitant appearance of an aspartic acid spot. In Fig. 1 (left), the relatively weak spot due to aspartic acid (No. 2) compared to asparagine (No. 6) suggests that the virus protein contains more asparagine than aspartic acid. In the acid hydrolyzate (middle) the aspartic acid spot (No. 2) has been suggested by the conversion of asparagine to aspartic acid.

A spot in the position usually occupied by glutamine was found in chromatograms of aliquots taken during the early stages (before the 7th day) of incubation. Since confirmatory tests were not made, position alone may not be a sufficient criterion for identification; there remains the possibility that the spot may have been due to the presence of a peptide in the incomplete hydrolyzate. On the other hand, glutamine, being a labile compound, may have been destroyed during the prolonged incubation.

All of the amino acids previously reported to be constituents of tobacco mosaic virus protein have been identified by paper partition chromatography. In addition, the presence of one amide, asparagine, has been demonstrated and the probable occurrence of a second, glutamine, has been suggested.

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Effect of Hyaluronidase on the Subcutaneous Absorption of Electrolytes in Humans[1]

Gilbert B. Forbes, Robert W. Deisher, Anne M. Perley, and Alexis F. Hartmann

Department of Pediatrics,
Washington University School of Medicine,
St. Louis

Although observations have been made in animals on the effect of testicular hyaluronidase on the subcutaneous absorption of isotonic saline (*7*), diodrast (*9*), diphtheria antitoxin (*6*), and plasma proteins tagged with radioiodine (*2*), studies on humans have been limited to measurement of the speed at which large subcutaneous infusions can be given. Whereas direct observation of the behavior of intradermal wheals (*5, 8*) is possible in humans, exact data on subcutaneous injections are more difficult to obtain. Since hyaluronidase is currently being used to facilitate the subcutaneous administration of

[1] This investigation was aided by a grant from the Children's Research Foundation.

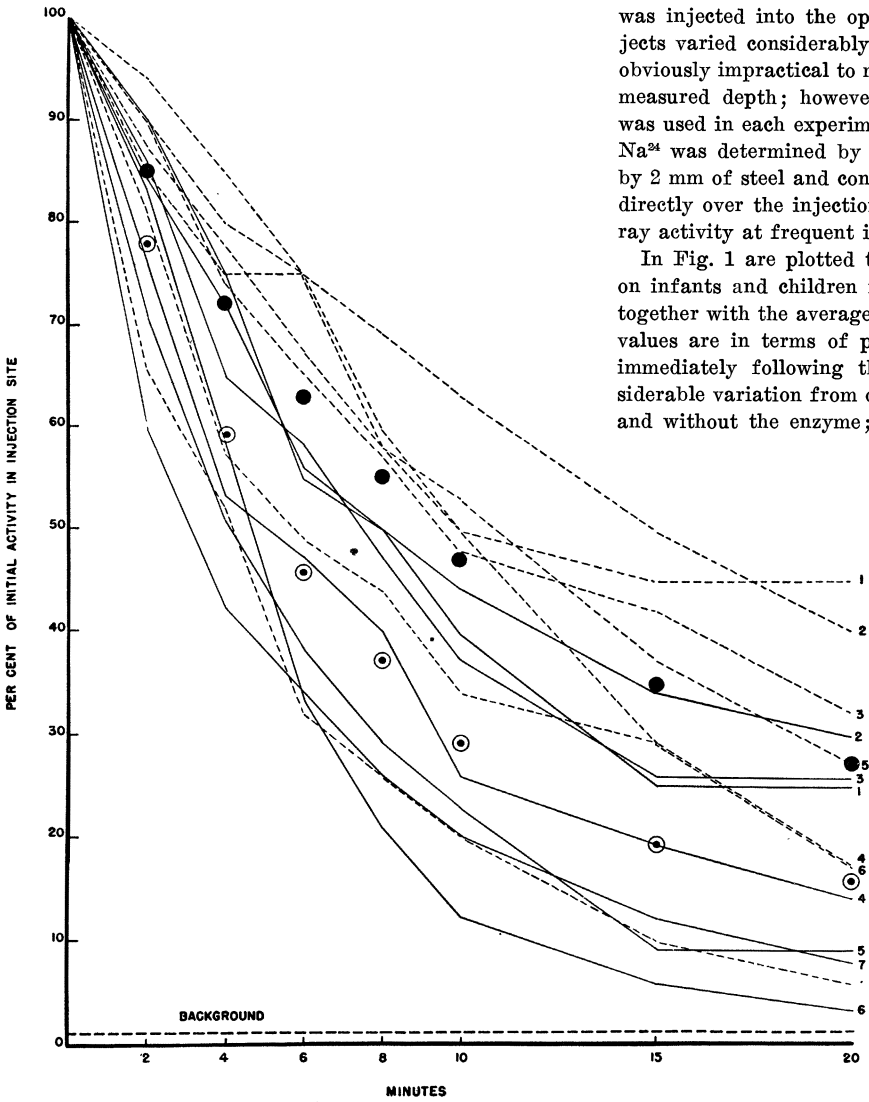

FIG. 1. Rate of disappearance of Na^{24} in isotonic saline from subcutaneous injection site, Control – – –, average ●: hyaluronidase ———, average ⊙. The numbers refer to individual subjects.

fluids to infants and children (*4, 5, 8*), it seemed advisable to quantitate its action on the absorption of electrolytes. The following experiments were carried out with 0.9% NaCl containing radiosodium (Na^{24}).[2] Observations were made on the rate of disappearance of Na^{24} from the injection site by means of an externally placed Geiger counter, and on the serum Na^{24} concentration following subcutaneous injection.

In the first group of experiments, 0.5 ml of saline solution containing 0.5–1.0 μc of Na^{24} was injected into the subcutaneous tissues of one forearm of the subject; a similar amount of Na^{24} to which had been added 25 turbidity reduction units of bovine testicular hyaluronidase[3] was injected into the opposite forearm. Since the subjects varied considerably in weight and physique, it was obviously impractical to make the injections at a constant measured depth; however, a similar injection technique was used in each experiment. The disappearance rate of Na^{24} was determined by placing a Geiger tube, shielded by 2 mm of steel and connected to a counting rate meter, directly over the injection site and recording the gamma ray activity at frequent intervals.

In Fig. 1 are plotted the results of seven experiments on infants and children in a normal state of hydration, together with the averages for all the subjects. Ordinate values are in terms of percent of the activity observed immediately following the injections. There was considerable variation from one subject to another both with and without the enzyme; however, in any given subject the addition of hyaluronidase definitely hastened the disappearance of Na^{24} from the injection site. The advisability of using each subject as his own control is apparent, for in some of the subjects the disappearance rate of Na^{24} was at least as great in the control injections as it was in others after addition of the enzyme. Furthermore, use of the enzyme produced different degrees of acceleration in the disappearance rate in different subjects.

In contrast to the definite effect noted in the normally hydrated subject, hyaluronidase did not appear to accelerate the subcutaneous absorption of Na^{24} in children with hypoproteinemic edema. In keeping with earlier studies on the disappearance of intradermal wheals (*1*), the few preliminary observations which we have made suggest that Na^{24} is absorbed somewhat more rapidly in the edematous subject as compared with the normal and that the disappearance curves of the control and enzyme injections are very similar.

In order to show that Na^{24} actually enters the blood from the subcutaneous tissues more rapidly under the influence of hyaluronidase, serum levels were followed in several normal subjects. In these experiments 2 μc of Na^{24} per kg body weight was injected into the thigh region by the method described by Barnett (*3*). The total volume of isotonic saline used was 4 ml. Serum samples were dried in metal dishes and assayed with a mica end window Geiger tube connected to a scaling circuit. In Fig. 2 are plotted the data from four subjects, each of whom received control and enzyme injections (150 units)

[2] Na^{24} was prepared in the Washington University cyclotron through the courtesy of A. A. Schulke and A. B. Phillips.

[3] Hyaluronidase ("Hyronase") was kindly supplied by the Schering Corporation, Bloomfield, New Jersey.

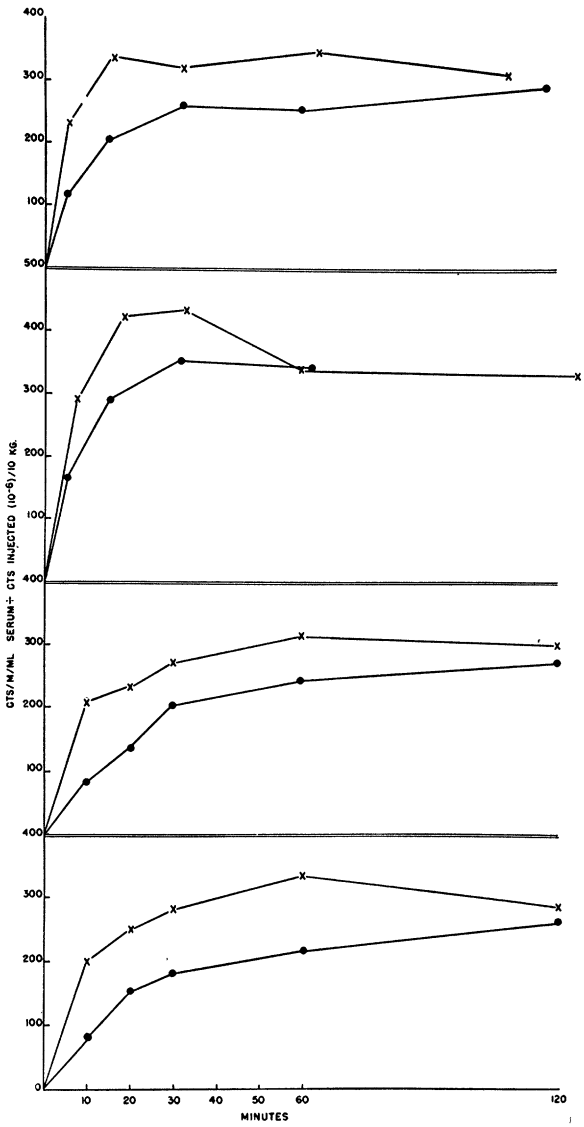

FIG. 2. Rate of serum uptake of subcutaneously injected Na^{24} in isotonic saline. Control ●——●, hyaluronidase ×——×.

on different days. Serum activity is expressed in terms of cpm/ml divided by counts injected (10^{-6}) per 10 kg body weight after correction for decay. Here, too, there was considerable variation in rate of absorption from one subject to another, despite correction of the data to constant body weight, but in each instance the serum Na^{24} value rose more rapidly when hyaluronidase was used.

From these observations, it is apparent that in the normally hydrated human subject, the rate of absorption of sodium ion from small volumes of subcutaneously injected fluid is enhanced by the addition of hyaluronidase.

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The Light Reaction in the Bleaching of Rhodopsin[1]

George Wald, Jack Durell, and Robert C. C. St. George

Biological Laboratories of Harvard University, Cambridge, Massachusetts

The absorption of light by rhodopsin in the retina results in both the bleaching of the molecule and the excitation of rod vision. One could conclude at once that the bleaching of rhodopsin is the source of visual excitation, were it not a composite process. It consists of an initial photochemical change, followed by relatively slow thermal —i.e., "dark"—reactions. The light process converts rhodopsin to a highly unstable orange material (Lythgoe's "transient orange"), which breaks down in light or darkness to a yellow mixture of retinene$_1$ and protein (Lythgoe's "indicator yellow") (*6*, *8*). In the retina these substances are involved in further changes, but in ordinary solutions of rhodopsin this is all that occurs.

The first product formed by the action of light on rhodopsin is removed so rapidly that early attempts to measure its spectrum led only to approximations (*7*, *8*). In 1941, however, Broda and Goodeve reported an experiment that appeared to isolate the light reaction (*2*).

These workers prepared solutions of rhodopsin in mixtures of glycerol and water (3:1) such as Kühne had examined more than 60 years before (*3*). On cooling to −73° C such solutions vitrify. The absorption band of rhodopsin was reported to shift about 10 mμ toward the red and to become much narrower in shape. On exposure to light at −73° C, the absorption maximum moved about 5 mμ toward the blue, and fell some 12 % in height. This was the light reaction. The photoproduct remained stable at the low temperature. It did not appear to vary in spectrum between pH 6 and 9, confirming an earlier conclusion of Lythgoe and Quilliam concerning transient orange. On warming to room temperature in the dark it decomposed spontaneously to indicator yellow.

We have reexamined this behavior of rhodopsin at low temperatures. Spectra were measured with the Beckman spectrophotometer in a specially designed Dewar flask, silvered except for a window. The rhodopsin solution, well buffered in a glycerol-water mixture (2:1) was suspended inside the vessel in a quartz cell over liquid air or solid carbon dioxide. The temperature of the solu-

[1] Supported in part by a grant from the Medical Sciences Division of the ONR. Low temperature experiments performed by Mr. St. George, those on rhodopsin films by Mr. Durell. Preliminary tests on rhodopsin films were also made by Lionel Jaffe.