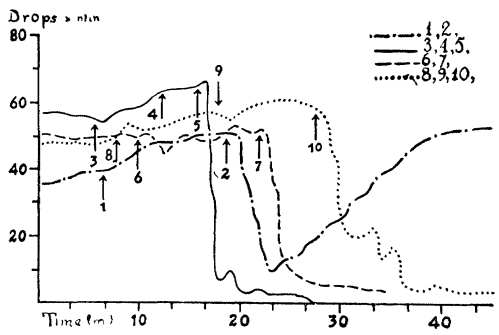
to show that a vasoconstrictor and hypertensive substance can be produced not only by incubation with renin but also by peptic digestion of hypertensinogen.

0.5 cc of a standard preparation of hypertensinogen[2] or renin activator[3] in hydrochloric acid (pH = 2 to 6) were incubated at 38° for 15 min. with 1 to 2 mgr of commercial or purified pepsin (Merck) in 0.1 cc of dest. water. A buffer solution of m/5 phosphate of pH = 7.2 was added. A vasoconstrictor effect of great intensity was observed in the Laewen-Trendelenburg preparation of the giant Chilean frog Calyptocephalus Gayi. We obtained also in cats a very remarkable rise of arterial pressure with hypertensinogen incubated with pepsin and conveniently concentrated and purified.[4]

Subsequently we were successful in obtaining a vasoconstrictor substance from different proteins (casein, fibrin, serumalbumin and ovalbumin) incubated with pepsin under conditions identical with those described above for hypertensinogen. The vasoconstrictor effect was always noticeable, though less than with pepsin-incubated hypertensinogen or with hypertensinogen incubated with renin (Fig. 1).

The physiological and chemical properties of the vasoconstrictor substance produced by incubation of proteins with pepsin also resemble those of hypertensin as described by Houssay: the substance is thermostable, soluble in water and strong alcoholic solutions, insoluble in ether, it dialyzes easily through the Cellophane membrane, it is precipitated by phosphotungstic but not by trichloroacetic acid. Trypsin destroys the vasoconstrictor substance produced by pepsin in a similar way as hypertensin is destroyed. Our substance behaved towards hypertensinase[1] also in a way similar to hypertensin: the substance was, like hypertensin, inactivated when mixed with renal extracts (from pig, human, rat) at a neutral pH.

It has been shown by Schroeder[5] with angiotonin (Page) and by our former work[6] with hypertensin that these substances are inactivated by tyrosinase of mushrooms. Likewise we found recently that the vasoconstrictor substance as derived from proteins is destroyed by tyrosinase. This is in favor of the assumption that hypertensin has a phenolic function. Other new findings of ours also are in full agreement with this assumption. Whereas so many proteins when incubated with pepsin generated the vasoconstrictor substance the latter failed to appear when *gelatin* was subjected to incubation with pepsin.



EXPLANATION OF FIGURE

Perfusion of Laewen-Trendelenburg preparation of the giant Chilean frog with Ringer. Ordinates—number of drops per minute. Abscissa—time in minutes. Arrow indicates addition of different solutions. 1. Solution of casein (Hammersten, 3%) for 30 min. in HCl (pH = 5.5); no vasoconstrictor action. 2. Same but incubated with pepsin (Merck-Payr); vasoconstrictor action. 3. Hypertensinogen in HCl; no action. 4. Solution of pepsin (Merck-Payr); no action. 5. Hypertensinogen incubated with pepsin (pH = 4.5); vasoconstrictor action highly pronounced and more stable than in 2 (pepsin-casein). 6. Pepsin in HCl; no action. 7. Hypertensinogen incubated with pepsin (pH = 6); highly pronounced vasoconstriction. 8. Purified gelatin (5%) incubated with pepsin; no action. 9. Same but double quantity added; no action. 10. Hypertensinogen incubated with renin; pronounced vasoconstrictor action.

Conclusions: A substance similar to hypertensin as to physiological, physical and chemical properties can be derived from hypertensinogen by incubation with pepsin. This substance is probably a polypeptid with a phenolic function and it is very likely that this applies also to hypertensin. The term "pepsitensin" seems appropriate for the new substance.

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IN VITRO CULTIVATION OF THE STREET VIRUS OF RABIES

SUCCESSFUL *in vitro* cultivation of the virus of rabies has been reported by Kanazawa (1936 and 1937)[1,2] employing a medium consisting of rabbit embryo brain tissue suspended in Tyrode solution,

[1] Kanazawa, *Jap. Jour. Exp. Med.*, 14: 519, 1936.

[2] Kanazawa, *Jap. Jour. Exp. Med.*, 15: 17, 1937.

without serum; by Webster and Clow (1936 and 1937)[3,4] in a medium consisting of mouse and chick embryo brain tissue suspended in serum Tyrode; and by Schultz and Williams,[5] who confirmed the latter's work. Kanazawa and Schultz and Williams employed a fixed rabbit strain while Webster and Clow employed three strains originating as follows: one strain from a skunk which was passed through six mouse brain passages, and two dog brain strains passed through eighty-eight and eight mouse brain passages respectively before initiating cultures. Direct *in vitro* cultivation of the street virus of rabies has not been reported.

The source of our infectious material was obtained as follows: About three months after a young man was bitten by a stray dog on the ring finger of his left hand, he developed symptoms of rabies and died in a typical attack. Examination of the brain showed Negri bodies. The brain was preserved in 50 per cent. glycerine. (This will be designated as strain A.)

The second strain was obtained from a rabid dog which was killed after paralysis developed. Examination of this brain showed Negri bodies. The brain was preserved in 50 per cent. glycerine. (This will be designated as strain B.)

Each brain specimen was treated as follows: Several pieces of brain were washed four times in Ringer's solution to remove the glycerine and then the material was ground in a mortar with alundum. A 10 per cent. aqueous suspension was made and centrifuged at 1,200 r.p.m. for ten minutes. The supernatant fluid was titrated for infectivity by inoculating serial dilutions—0.03 cc intracerebrally—into mice, and the rest of the supernatant fluid was employed for culture. The infectivity of strain A was 10^{-3}, killing mice after 14 days and Negri bodies were demonstrated in the brain (Sellard's stain).

Strain B was infectious in a dilution of 10^{-2}, killing mice in 9 to 12 days, with the presence of Negri bodies in the brain.

The medium employed for culture was essentially the same as has previously been described[6,7,8,9] for the cultivation of the virus of fowl plague, vaccinia and measles. Three cc of Tyrode solution and 0.5 cc of unfiltered monkey serum was placed in a 50 cc Erlenmeyer flask. To this was added four drops of a ten-day chick embryo cell suspension prepared with the Fisher press, and 1 cc of virus material and three drops of chicken plasma. The contents of the flask were gently shaken and stoppered with a rubber stopper. Cultures were maintained at 37° C. for three to four days.

After twenty-four hours the plasma clot was found floating on the surface of the liquid medium. Sections made of the clot show proliferating cells, in contrast to the non-proliferating cells which exist in a suspended cell medium. One of us[6,7] had previously shown that the virus of fowl plague and the virus of vaccinia grow much better in the presence of proliferating cells than they do in the presence of suspended cells. The experiments of Feller, Enders and Weller[10] bear on this observation.

Transplants are made by grinding the entire culture in a grinder with alundum and transferring 1 cc of the supernatant material to a new flask of media. Strain A was maintained through eleven transplants while Strain B was maintained through nine transplants.

During the course of these experiments, various transplants were inoculated intracerebrally into mice to determine the presence of virus. With strain A, mice died in from seven to eleven days, showing the presence of Negri bodies in the brain. The brain of a mouse that died after the inoculation with the eighth transplant (strain A) was titrated for infectivity. A dilution of 10^{-5} killed mice in eight days and the brains were Negri positive.

Titration of the clot of the eleventh transplant (strain A) killed mice in a dilution of 10^{-6} in eight and eleven days, but no Negri bodies were found. In a dilution of 10^{-5}, mice died in nine and eleven days and Negri bodies were demonstrated in the brains. The mice that died after inoculation with the lower dilutions were also Negri positive.

Titrations of the clot of strain B killed mice in twelve days in a dilution of 10^{-5} and the brains were Negri positive. Other mice inoculated with this dilution showed tremors on the thirteenth day and Negri bodies were demonstrated in the brain. Mice inoculated with lower dilutions, that died or were sacrificed when tremors were present, also showed Negri bodies in the brain. While strain B was infectious for mice in a dilution of 10^{-5}, this strain was not as virulent for mice as strain A.

We failed to cultivate the virus from another dog brain.

The street virus of rabies has been cultivated directly from the brain of a human case of rabies as well as from the brain of a rabid dog. The virus has been successfully cultivated through eleven and nine transplants respectively. The method of culture employed consists of whole chick embryo tissue instead of embryo brain tissue alone, as has hitherto been

[3] Webster and Clow, SCIENCE, 84: 487, 1936.
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[10] Feller, Enders and Weller, *Jour. Exp. Med.*, 72: 367, 1940.

used. The "floating clot" method has the advantage of providing a large mass of proliferating cells, which provide a great yield of virus. In view of this, experiments are being performed to determine whether these cultures can be employed as a vaccine.

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MECHANISM OF P-AMINOBENZOIC ACID ACTION AND THE PARALLEL EFFECTS OF ETHYL CARBAMATE (URETHANE)*

IN seeking a theoretical basis for the bacteriostatic effects of sulfanilamide, Woods and Fildes[1,2] first postulated that the drug competed with a structurally related molecule, para aminobenzoic acid (PAB), which was thereby presumed to occupy some essential rôle in the normal growth and metabolism of micro-organisms. The latter compound thus has a dual interest: as a possible intermediary in ordinary metabolism and as a possible site of sulfanilamide inhibitions. Numerous investigations seem to have provided evidence supporting both aspects of the original hypothesis, and to have greatly extended the biological significance of PAB.

It now appears widely accepted that PAB is not only a naturally occurring "essential metabolite,"[3] but an anti-sulfanilamide or a growth factor for diverse organisms, including chicks,[4] dermatophytes[5] and even autotrophic plants, *e.g.*, diatoms.[6] The same compound is thought to be concerned in lactation[7] and in pigmentation of hair.[4] Its anti-sulfanilamide effects on the growth of bacteria have been demonstrated *in vivo*[8] as well as *in vitro*.[1] Doubt has been expressed, however, that this anti-sulfanilamide effect is in the nature of a competitive action of the two molecules for the same receptor in the organism, since 1 molecule of PAB may antagonize 23,000 molecules of sulfanilamide. Before the above-mentioned interpretations become too deeply entrenched in the scientific literature and thought as fully correct, an alternative explanation for the mode of sulfanilamide action, as well as the stimulatory effects of PAB, should be considered.

Recent experiments in this laboratory have shown that *ethyl carbamate* (*urethane*) *as well as PAB may exert anti-sulfanilamide effects* on luminous bacteria. The results are more striking in relation to luminescence than to growth, although both are influenced. The structural similarities between the molecules of urethane and sulfanilamide are so remote as to rule out competitive action, and urethane could hardly be considered an "essential metabolite." It is a familiar principle, however, that narcotics and, indeed, poisons of many sorts, have stimulatory effects in low, and inhibitory effects in high concentration. All three of the above compounds—urethane, PAB and sulfanilamide—act in the manner of narcotics on luminous bacteria, stimulating growth and luminescence in low, while inhibiting in high concentrations.

Further evidence of the fundamentally narcotic action of PAB, sulfanilamide and urethane, quite apart from growing cultures, is found in their effects on washed cell suspensions. The intensity of luminescence is readily and reversibly reduced on the addition of any one of these or a host of other narcotics. Experiments with the luminescent luciferin-luciferase system, which can not be extracted yet from bacteria but can be obtained in purified preparations from *Cypridina*[9] have shown that the velocity constant of the reaction *in vitro* is retarded by urethane, PAB, sulfanilamide, sulfapyridine and sulfathiazol. The action is reversible and clearly on the enzyme, luciferase. Over a wide range it is independent of the substrate (luciferin) concentration.[10] Thus, the inhibitory effects of PAB, urethane and sulfonamides appear to be definitely related to those of narcotics in general. Recent work with hydrostatic pressure and temperature[11] has opened a new approach to the study of the basic mechanism involved.

The stimulatory action of narcotics in low concentration is not easy to explain. In the present connection, the point to be emphasized is that the stimulatory effects of one narcotic may antagonize or completely overcome the inhibitory effects of another that is simultaneously present. If the inhibitant is sulfanilamide, the antagonist is naturally looked upon as "anti-sulfanilamide." The anti-sulfanilamide action of both urethane and PAB might well belong in this category. The molecular structure of the antagonistic narcotics need not be closely related, as would be required for competitive inhibition in the physicochemical sense. The action of urethane and of nembutal in preventing death from sulfonamide overdosage of animals[12] lends support to the view ex-

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