immunity when inoculated intracerebrally or intraperitoneally into cotton rats.

Neutralization tests with serum-virus mixtures incubated for 1 hour at 37° C. and then kept in the icebox overnight were performed by inoculating cotton rats and hamsters intranasally. Sera from hyperimmunized cotton rats, hamsters and rabbits gave definite neutralization of the agent. Partial or irregular neutralization was observed with sera of human beings convalescent from atypical pneumonia, with sera of cotton rats inoculated once only or of guinea pigs inoculated repeatedly with cotton-rat adapted strains, and with sera from rabbits immunized with human lung infectious for cotton rats.

By cross-inoculation and neutralization tests antigenic relationships between 6 established strains were demonstrated. Cotton rats immunized by two successive intranasal inoculations with adapted strains were solidly immune to reinoculation with a specimen of infectious human lung which produced marked lesions in the controls. Cotton rats immunized with human material were partially resistant when tested with adapted strains.

During the course of serial passages from cotton rats which developed lung lesions on the first inoculation, two strains not antigenically related to those just described were obtained. These two "aberrant" strains may have been carried by the cotton rats and had apparently replaced the agent present in the first passages.

The appearance of non-bacterial lung lesions in cotton rats after inoculation of material from cases of atypical pneumonia suggests that a virus-like agent was transmitted and established by serial passage. The strains adapted to cotton rats were related to the agent in human material by cross-immunity tests. This agent, which is presumably a filterable virus, differs from the psittacosis-like virus previously described[3] and also from other known viruses which can infect cotton rats by the intranasal route. At present the evidence for the causal relation of this agent to the most common form of atypical pneumonia must be considered incomplete because of irregularities in the neutralization tests, particularly those with human serum. Further investigations on the influence of the amount of the infecting dose on the neutralization test are in progress.

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[3] M. D. Eaton, M. D. Beck and H. E. Pearson, *Jour. Exp. Med.*, 73: 641, 1941.

DESTRUCTION OF HYPERTENSIN AND PEPSITENSIN BY AN AMINOPEPTIDASE OBTAINED FROM YEAST

THE vasoconstrictor properties of hypertensin (angiotonin) and pepsitensin—a substance formed by the digestion of proteins with pepsin—can be entirely destroyed by an aminopeptidase (a.p.) enzyme obtained from yeast and purified by the Johnson method.[1]

These two hypertensive substances incubated with that enzyme at 38° and neutral pH. lose their vasoconstrictor properties in a few minutes.

Approximately 0.01 cc of the final purified solution obtained from 2 kg of compressed yeast destroys them after 5- to 10-minutes incubation (2 or 3 units of hypertensin or pepsitensin). The degree of destruction of these two products under the influence of the enzyme was controlled by the method previously described, using the Loewen Trendelenburg test and the arterial pressure of the cat.[2]

The mixture of hypertensin or pepsitensin with the enzyme was injected after different periods of incubation and the vasoconstrictor or pressor effects obtained were compared with those produced by an equal dose of substrate and the enzyme mixed immediately before injecting. Sometimes as a means of controlling the results, a mixture of enzyme was used, incubated for the same length of time, and previously inactivated by boiling with the vasoconstrictor substance (Fig. 1).

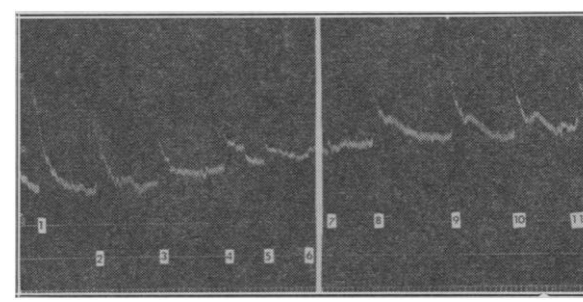

FIG. 1. Arterial pressure increase produced by: 1: 0.4 cc hypertensin; 2,3,4,5,6: 0.5 cc hypertensin incubated 5′ with 0.0025, 0.005, 0.01, 0.02 and 0.03 cc of a.p. 676 F respectively 7: 0.5 cc water with 0.03 cc a.p. 672 F 8,9,10,11: 0.5 hypertensin.

Titration of polypeptid nitrogen during incubation showed a progressive and considerable diminution. A similar result was obtained when renal hypertensinase acted upon hypertensin or pepsitensin.

The hydrolytic activity of yeast enzyme on synthetic substances allows one to classify this enzyme as a.p.[3] Hypertensinase extracts of pig kidneys, purified by

[1] M. J. Johnson, *Jour. Biol. Chem*, 127: 575, 1941.
[2] H. Croxatto and R. Croxatto, SCIENCE, 42: 101, 1942.
[3] J. S. Fruton, G. W. Irving, Jr. and M. Bergmann, *Jour. Biol. Chem.*, 141: 763, 1941.

acetone precipitation, and yeast enzymes hydrolyze l-leucylglycine. Hypertensinase and a.p. differ in their action on dl-methyl leucyldiglycine in that the former hydrolyze this polypeptide, while a.p. has no action. This effect may be due to the co-existence of other enzymes contained in the kidney extract, among others renin, which could not be completely separated (Table 1)[4].

TABLE 1

HYDROLYTIC ACTION OF a.p. OF YEAST AND OF HYPERTENSINASE ON SYNTHETIC SUBSTRATES

Enzyme	Undiluted enzyme cc	Time of incubation minutes	Hydrolysis, per cent. dl leucyl-glycine	dl M leucyl-diglycine
a.p. of yeast				
656 I	0.3	30	100	0
619 I	0.1	30	100	0
672 E	0.01	30	100	0
672 F	0.01	30	100	0
672 F	0.002	30	67	0
672 F	0.001	30	33	0
Hypertensinase				
655 II	0.1	180	100	33
655 II	0.1	30	100	18
655 I	0.1	30	100	38
655 I*	0.01	30	30	16
655 II	0.01	30	90	41
655 II	0.01	30	90	=
655 II†	0.01	30	0	=
655 II	0.03	30	100	31
655 II	0.02	30	=	=
655 II	0.02	30	=	=
655 II	0.10	30	=	=
655 II	0.03	30	=	=

* 655 I is a less concentrated product than 655 II.
† The enzyme was previously boiled. 100 per cent. means the total hydrolysis of a peptidic bond. 619 I contains more than 1,000 U. per gr of N. 655 II contains 425 U. per gr of N. 672 F contains 3,000 U. per gr of N. (Johnson) (1). a.p. hydrolyzes 100 per cent. leucyldiglycine. Chloracetyl-tyrosine and carbobenzoxyglycyl-l-sarcosine are not hydrolyzed by 655 II.

Moreover the a.p. acts like hypertensinase extracts, whether aerobically or anaerobically; it does not destroy tyramine and its effects on adrenalin are slight or nil.

The destruction of hypertensin and pepsitensin by a.p. establishes a similarity between both vasoconstrictor substrates which is added to those already described.[5] Their polypeptid nature is confirmed and the supposition that these vasoconstrictor substances possess a free NH_2 radical is strengthened. Hypertensinase activity of renal extracts may also be attributed to the existence of a.p. enzyme contained in kidney tissue.

The fact that renin acts as a hydrolytic enzyme of proteinase character (like pepsin)[6] places the cathepsins in a very important position in the problem of experimental hypertension.

[4] Our sincerest thanks to Dr. M. J. Johnson and Dr. M. Bergmann for their kindness in offering the polypeptides used in this paper.

[5] H. Croxatto and R. Croxatto, *Soc. Argent. Biol.*, 17: 439, 1941.

[6] R. Croxatto, H. Croxatto and J. Sorolla, *Rev. de Med. y Aliment. Chile*, 5: 135, 1942.

Conclusions: An a.p. enzyme separated from yeast inactivates both hypertensin and pepsitensin by a process of hydrolytic destruction. Purified hypertensinase containing renal extracts show a.p. activity.

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THE OCCURRENCE OF INTRAVASCULAR AGGLUTINATIONS IN AVIAN MALARIA[1]

INTRAVASCULAR agglutinations or clumps have been described by Knisely and co-workers as occurring in experimental monkey malaria and in various clinical conditions in man. The development of the Knisely quartz rod micro-illuminator has for the first time made possible an adequate study of histophysiology and histopathology of the circulation.

Using a quartz rod micro-illuminator the pathological changes of the circulation were studied in canaries infected with *Plasmodium cathemerium*.[2] The circulation was studied in eight infected and two normal canaries by placing the tip of the rod beneath the dorsal surface of the wing web. The canary was held in place with a specially designed holder which allowed exposure of a wing web ventral side up. A drop of mineral oil placed on the epithelium of the web facilitated visualization of the circulation at 96 × magnification.

All experimentally infected birds developed extensive infestations with parasite counts up to 67 per cent. and all but one died. As the parasite count began to rise progressive pathological changes were noted in the peripheral circulatory tree.

The initial changes were characterized by a loss of "streamlining" of flow, a slowing of the flow rates as indicated by a change in the contour of the parabolic fronts and transient stickings of white blood cells to the endothelial lining of the veinules. These changes were soon followed by evidences of early tissue damage such as plasma leaking with a spreading and rounding of fat cells due to an increase in interstitial fluid. The white cells became plastered to the endothelium in ever-increasing numbers and stuck with increasing cohesiveness as the infection progressed.

These initial changes, together with an increasing

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[2] The canaries were given a standardized infection through the courtesy of Dr. Redginald Hewitt, malariologist for the Tennessee Valley Authority.