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

[1] This is a preliminary report of observations in avian malaria supported by the Tennessee Valley Authority malarial research program at the University of Tennessee School of Medicine. The author wishes to acknowledge the courteous suggestions and counsel extended to him by Dr. Melvin H. Knisely, of the Department of Anatomy, University of Chicago.

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

parasitization, soon led to the development of more profound intravascular pathology, namely, the formation of sticky masses or clumps of red cells. These clumps or agglutinations were seen first only in the venous stream, were small (three to four red cells) and possessed but little intrinsic cohesiveness. As these clumps flowed into larger veins the shearing forces to which they were exposed broke them up. During the early stages of clump formation transient thromboses occurred and the flow rate was markedly retarded to sluggish in many areas.

In the subsequent 24 to 48 hours the usual picture was that of progressive intravascular clumping with the formation of larger agglutinated masses, more permanent thromboses and increasing tissue damage. The clumps or agglutinations now could withstand intravascular stresses as they circulate throughout the organism, appearing occasionally in arterioles. Streamlining was completely disrupted even in the large veins draining the area under observation. The viscosity of the plasma increased. This was demonstrated by the appearance of resistance met by a free red cell as it turned over in its path down stream and entered successively larger currents. These intravascular changes together with the intermingling of broken red cell clumps, free red and white cells and a rare white cell clump reminded one investigator of "sludge." This term vividly pictures the very marked intravascular pathology little evidence of which would be found with routine autopsy methods.

The circulatory damage resulting from these intravascular pathological changes together with the increasing parasitization precipitated a stage of generalized clumping which represented a status of irreversible pathology progressive to eventual circulatory failure. The clumps or agglutinated red cell masses, which were initially formed by parasitized red cells only and later by both infected and normal red cells, were less fragile than previously and showed marked intrinsic cohesiveness. The clumps stuck to one another and would, therefore, stick to the phagocytes of spleen, liver and bone marrow. However, their great size presented a mechanical difficulty that the phagocytes could not overcome (this fact has been demonstrated by M. H. Knisely in unpublished data). White blood cells stuck to the endothelium in layers, plasma leaking and skimming were marked, the paste-like blood flowed very sluggishly and thromboses became numerous. There was a further increase in the viscosity of the plasma subsequent to the marked plasma leaking. These circulatory changes were regularly followed by the death of the bird within a few hours.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE PREPARATION OF A SUCRASE-FREE TAKA-MALTASE

TAKA-DIASTASE is, as is known, a mixture of different enzymes including maltose and sucrose-splitting factors.

Leibowitz[1] advanced the theory of a specific gluco-maltase in taka-diastase distinct from taka-sucrase.[2] This view was opposed by Weidenhagen,[3] who postulated the existence of only one maltase identical with gluco-sucrase. Leibowitz and Hestrin[4,2] found a way to differentiate between the two disaccharide-hydrolyzing capacities of the taka-diastase owing to the greater thermostability and acid stability of the maltose-splitting factor.

The present paper describes a simple method for preparing maltase free from sucrase from the commercial taka-diastase (Parke, Davis and Company). The lability of taka-sucrase to reducing agents $N_2S_2O_4$, reported in a previous communication,[5] is exploited for this purpose.

2 gr of the commercial sample of taka-diastase were dissolved in 15 ml distilled water in a Cellophane bag and the solution under toluene dialyzed at room temperature for one day against running tap water and one day against distilled water, which was changed several times, until the solution is free of reducing substances.

The dialyzed solution (about 30 ml) was filtered into a glass-stoppered flask and mixed with 0.3 gr of sodium hydrosulfite. After 24 hours at room temperature, the $Na_2S_2O_4$ is removed by dialyzing the solution in a Cellophane bag for one day against running tap water and one day against distilled water, which is changed several times.

The dialyzed solution, showing only maltase activity, was highly diluted. To concentrate, dialysis against a 45 per cent. dextrine solution according to the method of Guy E. Youngburg[6] was attempted, but this method proved generally impracticable because of the reducing substances diffused in the concentrated solution from the dextrine. We succeeded

[1] J. Leibowitz, *Zs. physiol. chem.*, 149: 184, 1925; J. Leibowitz and P. Mechlinsky, *ibid.*, 154: 64, 1926.
[2] Cf. Sh. Hestrin, *Enzymologia*, 6: 193, 1940.
[3] R. Weidenhagen, *Ergeb. Enzymforsch.*, 1: 169, 1932; *Zs. physiol. chem.*, 216: 255, 1933.
[4] J. Leibowitz and Sh. Hestrin, *Nature*, 141: 552, 1938; 143: 339, 1939.
[5] J. Feigenbaum, *Biochem. Jour.* (1942) in press.
[6] Guy E. Youngburg, SCIENCE, 94: 498, 1941.