

PROTECTIVE ENZYMES, CYTOTOXIC IMMUNE SERA, AND ANAPHYLAXIS.*

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The announcement by Abderhalden of his theory of protective enzymes and his practical application of this theory to the diagnosis of pregnancy has stimulated a large amount of investigation, the general aim of which is to establish the theory as a basis for the explanation of the pathology of various diseases. The large amount of literature¹ which has already accumulated indicates not only how eagerly the method has been investigated as a practical aid in the diagnosis of pregnancy, but also the way it has been utilized in the study of other conditions, as malignant tumors,² disturbances of internal secretion³ and in nervous and mental diseases.⁴

The value of these applications, representing, as they do, efforts at immediate practical use, can be determined only by the statistics of cumulative work and may be left for the present to the efforts of the clinician using the exact methods of the laboratory. The extension of the theory, however, to include such diverse conditions as pregnancy, malignant growth, and mental disease, suggests that for such general application, the underlying principle should be definitely established.

That the theory of protective enzymes applies to fats and carbohydrates as well as to proteins is the view of Abderhalden, who, after injecting these various substances into animals, found in the serum respective ferments for each. Such a demonstration is of unusual interest to both physiologist and pathologist, and if definitely confirmed, it is a most important addition to our knowledge of the methods by which the body protects itself against the parenteral introduction of foreign material. But if the theory is to be applied to explain the pathology of disease, it is essential to demonstrate specificity of reaction to proteins of diverse composition or at least to the peculiar proteins of certain organs. Abderhalden's views⁵ as to specificity have been offered chiefly in his papers on the test for pregnancy. In connection with this test, he discusses various odd results: (1) 5 cases of eclampsia, the serum of 4 of which digested liver tissue and that of the fifth, thyroid tissue; (2) the serum of a case of nephritis of pregnancy which digested liver and kidney tissue; (3) that of a case of myxedema, with suspected pregnancy, which digested thyroid tissue but not placenta and liver; and (4) a case of Basedow's disease, the serum of which digested thyroid and ovarian

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¹ For the general literature see Abderhalden, *Schutzfermente des tierischen Organismus*, Berlin, 1912; *Abwehrfermente des tierischen Organismus*, 1913.

² Abderhalden, *München. med. Wchnschr.*, 1913, 60, p. 411; also Markus, *Berl. klin. Wchnschr.*, 1913, 50, p. 776.

³ Munzer, *ibid.*, p. 777.

⁴ Fauser, *Deutsch. med. Wchnschr.*, 1912, 38, p. 2446; *ibid.*, 1913, 39, p. 304; also *München. med. Wchnschr.*, 1913, 60, p. 584.

⁵ *München. med. Wchnschr.*, 1913, 60, p. 462.

tissue, but not liver and testicle. These side reactions may, however, it is suggested, represent the action of ferments which have developed as a response to disease of the liver, ovary, and thyroid, respectively, or it may be that the sera of certain diseases (cancer, febrile disease, and diseases accompanied by exudate) contain, in large amounts, the products of tissue destruction. It is definitely stated, however, that after the parenteral injection of some blood-foreign materials, as, for example, carbohydrates, the ferments resulting are not always strongly specific. They may digest not only the material injected, but also closely related combinations of the same class. This lack of specificity would appear, however, to apply more especially to the carbohydrates and not to proteins, for, by the optic method, the effect of a specially prepared protein serum upon closely related proteins failed to show a general proteolytic action.

On the basis of Abderhalden's general theory that the presence of the chorionic cells, or the products of their destruction, in the blood stream is responsible for the development of the protective ferment of pregnancy, one would expect ferments to appear in the blood-stream following the injection of kidney, liver, foreign serum, or other protein, which would be specific for the cell, serum, or protein injected. Some experimentation on this point exists. Thus Abderhalden¹ found that the subcutaneous or intravenous injection of placenta or placenta peptone into animals (including males) caused the development in the blood serum of a ferment capable of digesting human placenta. Positive results were obtained in each of 3 dogs injected twice with human placenta peptone and tested after 8 days; also in 6 rabbits injected 4 times with the fresh juice of human placenta and tested after 6 days; and likewise in 2 guinea-pigs receiving extract of guinea-pig placenta. In making the injection the intravenous method was used and in many instances the injections were made on successive days. Such experiments, in some of which the serum of animals receiving guinea-pig placenta was tested with human placenta, naturally bring up the question of specificity and also the question whether or not the protective enzyme has any relation to the active body in a cytotoxic immune serum.

It was to test these two points that the present investigation was undertaken, and as one of us had had considerable experience with the production of nephrotoxic immune sera, and was moreover desirous of applying the theory of protective enzymes to the study of the problems of nephritis, the work was limited to a study of the reactions following the injection of kidney substance.

Aside from the observations of Abderhalden, little evidence based on experimental study is at hand. Heilner and Petri² found that experimental hematoma in the rabbit causes the appearance in the serum, after 6 hrs., of bodies capable of digesting rabbit's liver, placenta, muscle, and coagulated blood serum. Petri³ also injected rabbits subcutaneously with their own serum and the serum of other rabbits and found, after 15 mins. to 48 hrs., that the serum of rabbits so treated contained fer-

¹ *Op. cit.*; see also *Deutsch. med. Wchnschr.*, 1912, 38, p. 2160, and *Ztschr. f. physiol. Chem.*, 1912, 77, p. 249.

² *München. med. Wchnschr.*, 1913, 60, p. 1530.

³ *Ibid.*, 1913, 60, p. 1137.

ments for coagulated serum, muscle, and liver of the rabbit. In one experiment 0.1 c.c. of rabbit serum caused the appearance of ferments in 6 hrs. Similar observations were made on man with the injection of human serum, with like results. Frank, Rosenthal, and Biberstein¹ injected rabbits and dogs intraperitoneally and subcutaneously with 5 gm. of sheep's kidney. After 36 hrs. the serum digested the liver and kidney of the rabbit and the sheep. In a second similar experiment serum drawn after 2 days digested human placenta, sheep's kidney, and chicken liver; in a third experiment, human placenta was digested, but not sheep's kidney; in a fourth, both were digested. When the sheep's kidney was injected a second time after an interval of several days a serum was obtained which digested only sheep's kidney. This they consider a selective ferment for sheep's kidney. In another communication, Frank and Rosenthal² compare the hemolysis and precipitin tests of a sheep-rabbit system with the dialysis method of Abderhalden and reach the conclusion that the protective enzymes have no relation to the bodies (amboceptor, etc.) concerned in immunity reactions. Steising,³ after studying, in connection with Abderhalden's dialysis method, inactivated sera with and without addition of guinea-pig and human complement, comes to the opposite conclusion and suggests that the so-called protective ferments belong in the group known by immunologists as lysins. Abderhalden⁴ states that amboceptor and complement play no part in the ferment theory and that Steising's positive results were due to the large amount of ninhydrin reacting substances which are frequently found, on account of peculiarities of digestion, in the serum of herbivorous animals.

Abderhalden⁵ also presents evidence to show that intravenous injection into a rabbit of 3 c.c. of hemolyzed rabbit's blood causes the serum of the injected animal to digest rabbit's red cells, but not rabbit's serum proteins.

I. NEPHROTOXIN AND PROTECTIVE FERMENT.

Methods.—Abderhalden's method of demonstrating the protective ferment of pregnancy⁶ is now so generally known that it is not necessary to give his methods in detail. Of the two, the optic and the dialysis methods, the latter has been used in this investigation. Our procedure has been as follows:

In the work with kidney protein immunity, the usual method of developing a cytotoxin was used. Dog's kidneys were washed free of blood by passing, under ether anesthesia, many liters of normal salt solution through the abdominal aorta. Under aseptic conditions the renal cortex was finely ground, mixed with salt solution, and injected into the peritoneal cavity of rabbits in amounts of 2–3.5 gm. For each treatment the material was freshly prepared. Some animals received a single injection; others 2–5 injections and after varying periods of time the animals were bled and the digestive action of the serum upon kidney and other tissues was tested. For the dialysis, Schleicher and Schüll diffusion sacs (Nos. 579 and 579A) which had been tested against peptone and normal serum were used. The kidney substance for the digestion tests was obtained in the same way as that used for the injections and after boiling and testing according to Abderhalden's technic, it was broken up into small

¹ *Ibid.*, 1913, 60, p. 1594.

³ *Ibid.*, 1913, 60, p. 1535.

² *Ibid.*, 1913, 60, p. 1425.

⁴ *Ibid.*, 1913, 60, p. 1641.

⁵ *Ibid.*, 1913, 60, p. 1703.

⁶ Williams and Pearce, *Surgery, Gynecology and Obstetrics*, 1913, 16, p. 411.

granular masses, again tested, and then placed in distilled water to which chloroform and toluene had been added and was kept in the refrigerator at a temperature a little above freezing. This material was boiled and tested before each series of observations. When the amount of serum obtained was sufficient, at least 8 sacs were used in each experiment, thus, serum of injected rabbit, (1) alone, (2) plus dog's kidney, and (3) plus dog's liver; normal rabbit serum, (4) alone, (5) plus dog's kidney, and (6) plus dog's liver; (7) kidney alone, and (8) liver alone. In some instances, when the amount of serum was small, as in the case of successive bleedings of the same animal, all these tests were not possible. The amount of serum used was 1.5 c.c. and of liver or kidney tissue, 1 gm. The sacs were kept at a temperature of 37.5° C. for 18-20 hours. The ordinary 50 c.c. centrifuge tube was found to be a convenient container for these sacs, as, in addition to the sac, it holds 20 c.c. of distilled water comfortably, with the sac in an upright position, and with the fluid within and without the sac at about the same level. Toluene was used freely within both tube and sac. For the demonstration of the products of digestion, ninhydrin (triketohydrindenhydrate) was used according to Abderhalden's directions.

In the first series of experiments, rabbits received a single injection of renal tissue and the serum of each was tested at various intervals after injection. The satisfactory results in this group are shown in Table 1.

TABLE 1.
EXPERIMENTS WITH SERA OF RABBITS RECEIVING A SINGLE INJECTION OF RENAL TISSUE.

EXPERIMENT No.	RABBIT No.	DAYS AFTER INJECTION	KIDNEY SERUM			NORMAL RABBIT SERUM			DOG'S KIDNEY ALONE	DOG'S LIVER ALONE
			Alone	With Dog's Kidney	With Dog's Liver	Alone	With Dog's Kidney	With Dog's Liver		
1.....	2	2	†	++	o
2.....	2	5	++
3.....	8	5	+	++	++	o
4.....	3	6	++
5.....	9	6	+	+	o	o	o	o	o
6.....	7	8	o	o	o	o	o	o	o	o
7.....	1	10	+	o	o	o
8.....	10	18	†*	o	o	o	o	o	o	o
9.....	1	33	+	+	o	o
10.....	3	37	++	+	o	o

† indicates faint reaction; +, well marked blue color; ++, deep blue color; o, no change.

* Positive result in this instance was due to a faulty sac.

The results differ widely. In Experiments 6 and 8 no digestive enzyme could be demonstrated 8 and 18 days, respectively, after injection. In Experiments 1, 2, 4, and 7, in which the amount of serum was insufficient for control tests with liver, an action on kidney tissue was evident, but in two of these (Experiments 1 and 7) the serum alone reacted faintly to ninhydrin. In Experiments 3, 5, 9, and 10, positive results were obtained with liver as well as kidney, and with one exception they were of the same degree of

intensity. In Experiments 2 and 10 the control normal sera gave faintly positive reactions; in Experiments 5, 6, 7, 8, and 9, they were negative. When an animal was used more than once the same reaction was obtained at each bleeding (see Experiments 1 and 2, 7 and 9, 4 and 10). The periods after injection at which positive results were obtained varied from 2 to 37 days.

In analyzing these results, one must keep in mind the many difficulties of the technic. The sacs go wrong so frequently that they must be tested against peptone and serum again and again and especially whenever an unexpected result occurs. Difficulty also arises from the presence of even small traces of hemoglobin in the serum and this complication cannot always be avoided, no matter how carefully the serum is collected. Again, as Abderhalden¹ has pointed out, and also McCord,² ninhydrin reacting substances, on account of the peculiarities of digestion in herbivorous animals, are frequently present in the normal serum of the rabbit. These possibilities of error, peculiar to the method, demand great caution in the reading of results, and that they are real difficulties is shown by the fact that of 23 tests made, only 10 were sufficiently free of possible error to be considered as satisfactory for insertion in Table 1. The greatest difficulty in interpretation has occurred naturally in those experiments in which the serum itself gave a reaction. Our rule has been, if such reaction is faint as compared to a strong reaction in tube containing serum and kidney, to consider the results as satisfactory in a comparative sense. If, however, the 2 tubes show reaction of nearly equal degree, the entire experiment is considered worthless. This procedure is in accord with the principles of the technic as laid down by Abderhalden,³ and seems justified by our own experience with normal control sera occasionally giving a faint positive reaction (see Experiments 2 and 10). With the exception of such comparable reactions all tests in which discordant results occurred have been ruled out. Even with these precautions, we present the above table with some hesitation, because of our doubt of the value of a method which does not give clean-cut controls.

¹ *Op. cit.*

² *Surgery, Gynecology and Obstetrics*, 1913, 16, p. 418.

³ *Op. cit.*

However, as presented, our results indicate that the injection into the rabbit of blood-free kidney of the dog results in the production of a ferment capable of acting upon dog's kidney *in vitro*. This ferment appears in the serum in our experience within 48 hrs. and is still present after 37 days. Inasmuch, however, as it acts with equal power upon dog's liver also, it cannot be considered as specific for the kidney. Occasionally the injection of kidney does not yield a ferment.

As Frank, Rosenthal, and Biberstein¹ report that 2 injections of kidney substance cause the appearance in the serum of a ferment having a selective action on the kidney, we continued our observation on animals receiving 2-5 injections of renal substance.

Here again we have met with many difficulties. Discordant results have been frequent; thus negative results have been obtained after 2-3 injections and positive results after 4-5 injections. When the first negative results after 2 injections were obtained, it was thought that protective enzyme production might be analogous to anaphylaxis, that is, that multiple injections might alter the enzyme production as they do sensitization in anaphylaxis. Later results showed, however, that this view was untenable. Nevertheless, it is difficult to understand why, with similar technic, a serum should be active after 4-5 injections and not always so after 2-3 injections, especially when positive results are readily obtained as a rule after one injection. We have no explanation to offer, but believe that the results in the negative experiments represent some error inherent in the method or some peculiarity in the reacting power of the animals (compare Experiments 6 and 8 of Table 1).

To illustrate this phase of our study the results obtained with the serum of the 2 rabbits receiving the greatest number of injections are presented in Table 2. The injections were made as in the earlier series, and repeated at intervals of 6-7 days. Rabbit 4 was bled 9 days, and Rabbit 12, 8 days after the last injection.

As regards the question of specific action these experiments indicate a more definite action upon kidney than upon liver, and in so far as a small number of observations are of value, support the view that multiple injections tend to a slight selective action.

¹*Op. cit.*

Another object of this investigation has been to determine whether a serum showing a digestive action on kidney *in vitro* has also nephrotoxic power *in vivo*, that is, to determine the question of the relation between protective enzyme and immune cytotoxin. Inasmuch as it has been shown by earlier workers¹ that a nephrotoxic serum may be readily produced by three or more injections of renal substance, the present study has necessarily been limited to the serum of animals which after a single injection developed the power of digesting kidney tissue *in vitro*.

TABLE 2.
SERA OF RABBITS RECEIVING MULTIPLE INJECTIONS OF DOG'S KIDNEY.

RABBIT No.	NO. OF INJECTIONS OF DOG'S KIDNEY	KIDNEY SERUM			NORMAL SERUM			DOG'S KIDNEY ALONE	DOG'S LIVER ALONE
		Alone	With Dog's Kidney	With Dog's Liver	Alone	With Dog's Kidney	With Dog's Liver		
4.....	4	0	+	†	0	0	0	0	0
12.....	5	†	++	+	†	†	†	0	0

Such sera have been injected intravenously into dogs and the urine of these animals examined for albumin and casts and finally their kidneys examined for histological changes. The sera selected were those of Rabbits 1, 8, and 9 (see Table 1), all of which were active against kidney tissue, 5, 6, and 33 days, respectively, after a single injection of kidney. These sera given intravenously to dogs in doses of 1-2 c.c. per kilo of body weight caused no disturbance of the kidney. The urine was always free of albumin and the kidneys normal histologically. It would appear, therefore, that the so-called protective ferment resulting from the injection of kidney is not identical with the active substance of nephrotoxic serum, or at least that if the latter is present, it is in such small amount as not to be demonstrable.

In addition to the above experiments in which dog's kidney was injected into the rabbit, 2 dogs received, respectively, 2 and 3 injections of dog's kidney and their serum was tested against dog's kidney *in vitro*. In the first of these the interval between injections was 5 days and the animal was bled 2 days after the second injection; in the other the intervals were 4 and 6 days, respectively, and the bleeding occurred one week after the third injection. Negative results were obtained. These results are not in accord with those of Abderhalden, who found ferments as the result of injecting dog's kidney into the dog. Comparison, however, is not exactly proper, as Abderhalden injected frequently into the circulation and used the optic method.

In connection with the experiments just described, a few experiments were made with the serum of animals suffering from experimental nephritis. The hypothesis upon which these experiments were based was that in animals with destruction of renal tissue, as that caused by a nephritic poison, protective enzymes might develop

¹ See Pearce, *Univ. of Penna. Med. Bull.*, 1903, 18, p. 557.



in the serum. That this rather far-fetched hypothesis has no basis in fact was shown by negative results with the serum of a rabbit in the fifth day of severe uranum nitrate nephritis and with that of a dog with a chronic nephritis due to multiple injections of uranum nitrate and potassium chromate.

In this connection it may be added that the attempts to apply Abderhalden's test to the clinical study of nephritis have given unsatisfactory results. Deutsch and Köhler¹ in a study of 22 sera from cases of chronic nephritis and amyloid kidney obtained a digestion of kidney tissue in 17; positive results were likewise obtained by the use of the same sera with placenta and thyroid. On the other hand, the serum of menstruating women in six instances also digested kidney. Lampé and Popazolu,² who studied the serum of several individuals with nephritis, found no evidence of specific action on kidney tissue. In so far as the problems of nephritis are concerned, Abderhalden's theory of protective enzymes as a means of investigation would appear therefore to be of little value.

II. ANAPHYLAXIS AND PROTECTIVE FERMENTS.

The theory that anaphylaxis depends upon a specific ferment developing as the result of the parenteral introduction into the body of a foreign protein has, since the work of Vaughan and his associates in this field, gained wide acceptance. Abderhalden's theory of protective enzymes, altho more comprehensive in its scope, naturally includes the lesser field of anaphylaxis and it is not surprising that early in his studies of the protective enzymes Abderhalden³ approached the subject of anaphylaxis from the point of view of ferment production as did also, a little later, Pfeiffer and Mita,⁴ and Gruber.⁵ Within the last year Abderhalden⁶ has published some results of the study of anaphylaxis which are of considerable interest.⁷ These are as follows: (1) sera from 12 guinea-pigs sensitized to egg-white, when mixed with antigen, showed digestive power by both optic and dialysis (biuret) methods; (2) similar sera, dialyzed alone, showed digestive products in only 1 of 6 sera tested; (3) serum of 6 guinea-pigs taken at intervals of 5 mins. to 1½ hrs. after the second injection (egg-white) and dialyzed gave negative results after 5 and 15 mins., while 4 taken after 30, 45, 60, and 90 minutes, respectively, were positive. In each test the serum (10 c.c.) was dialyzed against distilled water for 16 hrs. at 37° C. and the presence of products of digestion determined by the biuret reaction.

¹ *Wien. klin. Wchnschr.*, 1913, 26, p. 1361.

² *München. med. Wchnschr.*, 1913, 60, pp. 1423 and 1533.

³ *Ztschr. f. physiol. Chem.*, 1909, 61, pp. 199 and 426; 62, p. 243; also *Schutzfermente*, loc. cit.

⁴ *Ztschr. f. Immunitätsf.*, 1910, 6, p. 18.

⁵ *Ibid.*, 1910, 7, p. 762.

⁶ *Ztschr. f. physiol. Chem.*, 1912, 82, p. 109.

⁷ Compare also Zunz, *Ztschr. f. Immunitätsf.*, 1913, 17, pp. 241, 265, and 279.

These observations of Abderhalden's, in view of our experience with antirenal sera, led to the study of animals sensitized to a foreign serum.

Methods.—Dogs received in the peritoneal cavity a single injection of 5 c.c. of horse serum and after the lapse of 3 or more weeks the power of their serum to digest fresh and coagulated horse serum was tested both before and after the second, or intoxicating injection, which was always intravenous and consisted of 3–5 c.c. of the same horse serum. In the digestion experiments 2 c.c. of serum were used in each tube, dialysis was allowed for 18–20 hrs. at 37° C., and ninhydrin was used in all tests to detect the presence of the products of digestion.

As in the work with kidney sera, some difficulty was experienced on account of occasional slight hemoglobin staining of the sera and also because control sera sometimes gave as definite a reaction as the sera of treated animals. It is for these reasons that data cannot be given (see Table 3) concerning the activity of each serum both before and after the second injection. For the same reasons observations on several animals are omitted entirely. Whether or not the positive results with normal serum (not here presented) are due to the presence of proteolytic ferments in the serum of normal dogs¹ we will not discuss at this time.

TABLE 3.
DIGESTIVE POWER OF SERUM OF SENSITIZED ANIMALS BEFORE AND AFTER SECOND INJECTION.
BEFORE SECOND INJECTION.

DOG No.	SENSITIZED SERUM			NORMAL SERUM			HORSE SERUM ALONE
	Alone	With Fresh Horse Serum	With Coagulated Horse Serum	Alone	With Fresh Horse Serum	With Coagulated Horse Serum	
15.....	o	o	o	o	o	o	o
11.....	o	o	o	o	o	o	o
12.....	o	o	o	o	o	o	o
13.....	o	o	+	o	o	o	o

AFTER SECOND INJECTION.							
15.....	o	o	o	o	o	o	o
11.....	o	+	o	o	o	o	o
14.....	o	+	o	o	o	o	o

Table 3 shows the general results with the first group of animals studied. All sera "after shock" were obtained within periods varying from 4 to 10 minutes after the fall in blood pressure, as determined by kymographic tracing, had occurred. The results are fairly uniform, but are not in accord with those of Abderhalden in that negative results were always obtained before the second injection (except once with coagulated serum), and positive results in two of the sera after such injection. The dialysis of serum alone (2 c.c.) never gave positive results. The almost uniformly negative results with sera obtained before the second injection suggests the possibility of impermeable sacs, but as each sac was satisfactorily tested before and after each test with Witte's peptone and with blood serum the explanation is not tenable. There

¹ Compare Pincussohn, *Biochem. Ztschr.*, 1913, 51, p. 107.

is, however, one point of difference between Abderhalden's technic and ours. Abderhalden used the biuret test while we used ninhydrin. In our hands the biuret test has been most unsatisfactory. Another difference lies in the fact that Abderhalden used guinea-pigs sensitized to egg-white, while we used dogs sensitized to horse serum. The negative results before the intoxicating dose indicate that, by the method used, the serum was, contrary to Abderhalden's experience, without specific ferment. On the other hand, if "shock" following the second injection is the result of the digestion of the foreign protein by specific enzymes, the products of such digestion should be found in the serum. Such products ninhydrin failed to demonstrate when the sera obtained after "shock" were dialyzed without the addition of horse serum. The amounts used, however, were small (2 c.c.) and the serum was obtained always within 5-10 mins., while Abderhalden's positive results were obtained with 10 c.c. of serum from blood drawn at intervals of 30 to 90 mins. after the second injection. In a second series of observations, in order to cover these variations, we used larger amounts of serum (10-20 c.c.) obtained from blood drawn at intervals of 30, 60, and 90 mins. after "shock." These larger amounts were dialyzed alone and the dialysate tested with ninhydrin. Here, again, our work has been most unsatisfactory. The serum from the non-coagulable blood, obtained half an hour or more after shock, has in each of 5 experiments been more or less stained with hemoglobin and with every precaution in collecting and centrifuging we have failed to avoid this difficulty.¹ Dialysis of this serum has always given positive results, but in view of the presence of hemoglobin it has been impossible to decide upon their significance. In an attempt to determine whether the positive results in question are due to the presence of hemoglobin or the products of protein disintegration, we have tried to demonstrate the latter directly. We have removed, immediately after centrifuging the freshly obtained blood, all coagulable protein by heat and acetic acid, or by absolute alcohol and zinc chlorid, or, as we have found to be better, by combining both methods. The resulting filtrate evaporated to dryness, brought back by addition of distilled water to the original volume of serum, and neutralized, has usually given a negative reaction in the case of normal dog serum even tho it be slightly hemoglobin-stained. On the other hand, each of 3 sera obtained after shock and tested in this way gave a positive reaction. On the assumption that by this method all coagulable protein had been removed, these positive reactions could have been due only to the presence of the products of protein disintegration.

The method is not, however, without possibilities of error, one being the uncertainty of removal of all coagulable protein and the other being the destruction of ninhydrin in an excess of acetic acid. (This last source of error we have studied carefully and we have found it to be a very definite cause of trouble. It can be avoided, however, by neutralization of the fluid with weak sodium hydrate solution before adding ninhydrin.) These possible errors we believe have been adequately controlled. Nevertheless, our

¹ Serum from blood drawn 5-10 mins. after shock is occasionally hemoglobin-stained, but as a rule is not; after half an hour, however, it has been our experience that there is always a faint tingeing. For this we have no explanation.

results of the study of the serum of the dog after "shock," tho offered as corroborative of Abderhalden's observations on the serum of the guinea-pig after shock, are presented with some hesitation on account of the fact that our method of procedure differs from that of dialysis so widely as to be open to criticism.

In this connection it is of interest to recall that Auer and VanSlyke¹ failed, by direct determination of amino-nitrogen, to find an increased amount of protein cleavage products in the anaphylactic lung of the guinea-pig.

On the whole our results with the serum of anaphylactic animals are very unsatisfactory. The action on fresh horse serum of serum from the dog obtained before "shock" was not demonstrable in 4 experiments, altho in one a positive result was obtained with coagulated horse serum. After "shock," positive results were obtained with 2 of 3 sera in the case of fresh horse serum, but in none with coagulated horse serum. In none of these experiments did the serum (2 c.c.), when antigen was not used, give a positive reaction on dialysis. When, as did Abderhalden, we used larger amounts (10-20 c.c.) of serum obtained $\frac{1}{2}$ -1 $\frac{1}{2}$ hrs. after shock, positive results were obtained, but as the serum was hemoglobin-stained, the interpretation was doubtful. As, however, positive results were obtained with the filtrates of these sera after removing the coagulable protein, it would seem probable, despite certain possibilities of error, that the serum after shock does contain the products of protein disintegration. Yet the results are not sufficiently definite to afford proof of the view first elaborated by Vaughan and now adopted by Abderhalden that enzymes developing as the result of the parenteral introduction into the body of foreign protein constitute the essential basis of anaphylaxis. Inasmuch, however, as the suggestive results here given are in part comparable to those of Abderhalden, it is quite possible that some refinement of the method or a nearly allied method of greater accuracy may offer definite proof of Vaughan's very important and presumably correct hypothesis. But we are not in general accord with the results obtained by Abderhalden.

¹ *Jour. Exper. Med.*, 1913, 18, p. 210.

Setting aside the question of any relation which our results may or may not have to the theories of anaphylaxis, it is evident from the results in the first three experiments of Table 3 that the injection of a foreign protein is not always followed by the development of enzymes demonstrable by Abderhalden's dialysis method.

SUMMARY.

1. On the basis of Abderhalden's theory of protective enzymes and by the use of his dialysis method it has been shown that the serum of a rabbit receiving a single injection of kidney substance develops the power to digest dog's kidney *in vitro*, but has no effect upon the kidney of the dog when administered intravenously. Thus it would appear that the so-called protective enzymes are not to be classed with the immune cytolytins.

2. The digestive power of the serum which develops after the injection of kidney is not limited to the kidney but acts also upon the liver. This is true after one injection or after 4 or 5 injections. There is some evidence, however, after multiple injections of a tendency to a more definite effect on the kidney than on the liver.

3. A few attempts to demonstrate protective enzymes in the serum of dogs receiving dog's kidney and of animals with experimental nephritis have failed.

4. Attempts to demonstrate protective enzymes in the serum of dogs sensitized to horse serum have not been as successful as those of Abderhalden with the serum of the guinea-pig sensitized to egg-white. Negative results have been the rule before shock, and positive results, difficult of explanation, after shock.

5. Dialysis, alone, of small amounts (2 c.c.) of serum, obtained either before or 5-10 mins. after "shock" in dogs sensitized to horse serum, gives no evidence of the presence of the products of protein disintegration. Larger amounts (10-20 c.c.) taken $\frac{1}{2}$ -1 $\frac{1}{2}$ hrs. after shock give positive results after dialysis, but the interpretation of these is doubtful on account of the difficulty, under these circumstances, of obtaining serum free of traces of hemoglobin.

CONCLUSIONS.

The results of the injection of renal tissue support Abderhalden's general contention concerning protective enzymes but indicate a lack of specificity. On the other hand, our work with anaphylaxis, while suggestive, is not sufficiently definite to be used in support of the theory that the essential mechanism of anaphylaxis can be explained on the theory of the development of a protective enzyme. Finally, we wish to state frankly, that on account of the many difficulties which the technic of this method presents—and especially because of the frequent presence of ninhydrin reacting substances in the serum of normal animals—thus rendering exact control observation difficult, these results are presented with some hesitation. Moreover, without desiring to detract in any way from the importance of the underlying principle of Abderhalden's theory of protective enzymes as exemplified by his work on pregnancy, we urge caution as to hasty attempts to apply this theory as a general explanation of widely diverse conditions of altered physiology.