AMMONIUM SALTS AND THE ABDERHALDEN TEST *

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In an earlier communication¹ we have shown that placental substrate, when extracted with water or normal salt solution, yielded considerable quantities of ammonia nitrogen, and that positive Abderhalden (ninhydrin) reactions had been obtained with blood serum dialyzed with ammonium chlorid as a substrate.

It has also been demonstrated² that, contrary to the opinion expressed by Abderhalden and others, all ammonium salts, if sufficiently concentrated, react positively to ninhydrin. None, with the exception of ammonium-sodium-hydrogen phosphate, gave a positive reaction in a dilution as great as 0.05 mg. nitrogen per cubic centi-

TABLE 1.—NINHYDRIN TESTS WITH AMMONIUM PHOSPHATE AND GLUCOSE MIXTURE

Ammonia Nitrogen Content, Mg.	Glucose, Mg.	Result
0.28	2	Negative
0.35	2	+ (faint)
0.49	2	+
0.63	2	++
0.35	1	Negative
0.56	1	+ (faint)
0.70	1	+

meter, and the result here was so faint as to be negligible. It was, however, found that the addition of a reducing agent, pyridin, yielded positive reactions, even in such dilute solutions, with all ammonium salts. The method of conducting the ninhydrin test in these experiments differed from that of the Abderhalden technic in that 1 c.c. of 1 per cent. ninhydrin was used and the boiling was continued for twenty minutes.

In view of the fact that blood serum normally contains at least one reducing agent, glucose, in considerable quantity, it seemed worth while to perform tests with ammonium salts and glucose and to employ the technic of Abderhalden, wherein 0.2 c.c. of 1 per cent. ninhydrin is added to 10 c.c. and the mixture is boiled for one minute only.

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¹ From the Laboratory of the Illinois State Psychopathic Institute.

1. THE ARCHIVES INT. MED., p. 529.

2. Harding and Warneford: Jour. Biol. Chem., 1915, xxv, 319.

Qualitative tests of the normal salt extract from placenta substrate demonstrated the presence of considerable amounts of phosphoric acid and, since ammonium phosphate seemed to react especially readily with ninhydrin, it was decided to employ the neutral ammonium phosphate for this purpose. As a reducing agent we have used glucose. Table 1 gives the results of a series of tests in each of which the volume of ammonium phosphate-glucose solution was made up to 10 c.c.

Placenta substrate, which had been washed twice (the wash water was negative to ninhydrin), was extracted with normal salt solution for sixteen hours. The ammonia nitrogen in the extract was estimated as 0.62 mg. for each gram of placenta. Table 2 gives the results of boiling portions of the extract, made up in each case to 10 c.c. by the addition of water, with and without glucose, for 1 minute with 0.2 c.c. of 1 per cent. ninhydrin:

TABLE 2.—NINHYDRIN TESTS WITH NORMAL SALT EXTRACT OF PLACENTA SUBSTRATE AND GLUCOSE MIXTURE

Ammonia Nitrogen Content, Mg.	Equivalent Placenta Substrate, Gm.	Glucose, Mg.	Result
0.99	1.6	...	Negative
0.62	1	1	Doubtful
0.73	1.2	1	+

In the Abderhalden tests, as usually carried out, the substrate is washed four or five times before use and the amount of ammonia nitrogen which would be extracted in sixteen hours would be considerably less than with the substrate here employed, which had been washed with boiling water only twice. The weight of substrate used is generally from 0.25 to 0.5 gm. Furthermore, glucose is present in normal blood serum to the extent of about 1 mg. per cubic centimeter. From 1 to 1.5 c.c. are used for a test and the serum dialyzed into 20 c.c. of water, 10 c.c. of which are used for the actual ninhydrin reaction. This 10 c.c. can therefore rarely contain more than 0.5 mg. glucose.

In the results given above it was necessary to use the extract from 1.2 gm. placenta substrate with 1 mg. glucose in order to obtain a positive ninhydrin reaction following the Abderhalden technic, and there would therefore appear to be but little danger of error from this source, provided care is used in washing the substrate and too large quantities of serum and substrate are avoided.

In order to confirm this opinion we have carried out some dialysis tests with placenta substrate and a solution of glucose and others with blood serum and ammonium phosphate solution. In the former 0.5, 1, and 1.5 gm. placenta were placed in dialyzing thimbles with, in each instance, 1 c.c. of glucose solution containing 2 mg. glucose. There

were placed 20 c.c. distilled water in the tube outside the thimble and 10 c.c. of this was used after sixteen hours' incubation for the ninhydrin test. All gave a negative result.

In the second series blood serum from a normal person was used, some being drawn before breakfast and a second sample one hour after breakfast. The glucose content was determined by the method of Strouse, Stein and Wiseley³ to be 0.725 mg. per cubic centimeter before breakfast, and 1.189 mg. per cubic centimeter after breakfast. The results of dialysis tests were as given in Table 3.

TABLE 3.—NINHYDRIN TESTS WITH THE DIALYSATE FROM AMMONIUM PHOSPHATE AND BLOOD SERUM MIXTURE

	Serum, C.c.	Ammonium Phosphate in Mg. of Ammonia N	Result
Before breakfast.....	1.5	0.315	+ (very faint)
	1.5	0.49	+
	1.5	0.7	+
After breakfast.....	1.5	0.315	Lost
	1.5	0.49	+ (very faint)
	1.5	0.7	+ (slight)

The reaction with 0.7 mg. ammonium phosphate and serum taken before breakfast was very marked, more so than that with the same amount and after-breakfast serum. Since the latter contained a larger quantity of glucose, it seems probable that the positive reactions here depend on autolysis of serum and are not merely reactions between the ammonium salt and ninhydrin in the presence of a reducing agent, the glucose of the serum. This reaction is similar to that previously reported with ammonium chlorid as a substrate.

SUMMARY AND CONCLUSIONS

Ammonium phosphate will serve as an efficient substrate in the performance of the Abderhalden test.

Ammonium phosphate in dilute solution will give a positive reaction with ninhydrin, used according to the Abderhalden technic, in the presence of glucose.

Although ammonia nitrogen and phosphoric acid are present in the watery extract from placental substrate and glucose is present in the blood serum, the quantities are too small to give rise to error when the Abderhalden technic is followed.

3. Strouse, Stein and Wiseley: Bull. Johns Hopkins Hosp., 1915, xxvi, 211.