Conclusions.

1. The quantity of acetone bodies excreted by the normal individual, on an ordinary diet containing a sufficiency of carbohydrate, is influenced chiefly by the protein intake. On an ordinary diet 10–30 mg. are excreted daily.

2. The administration of quite small amounts of carbohydrate to the starving organism brings about a great reduction in the acidosis.

3. The administration of protein to the fasting organism causes a similar decrease, but this is neither so marked nor so rapid as in the case of carbohydrate.

4. The administration of glycerol under similar conditions also causes a very definite reduction in the acidosis.

5. The administration of fat to the starving organism increases the acidosis.

6. Administration of alcohol is without effect on the degree of acidosis.

7. The amount of acetone bodies in the urine during the first few days of starvation depends on the initial carbohydrate storage. These substances appear in abnormal amounts immediately the ratio of the fat to the carbohydrate burnt becomes greater than about 2 : 1. Immediately the ratio becomes less than this the acetone output is reduced to normal.

8. While a relatively large amount of carbohydrate is required to prevent acidosis, quite a small amount suffices to check it very markedly.

9. When acetone bodies are excreted in excessive amounts they are for the most part derived from fat.

10. Some evidence is put forward in favour of the possibility of fat being converted into carbohydrate in the body.

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YOUNG

XLV. THE FIXATION OF SALVARSAN AND NEOSALVARSAN BY THE BLOOD AFTER INTRAVENOUS INJECTION.

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(*Received October 27th, 1915.*)

It was found by Nierenstein [1908] that when serum was shaken with atoxyl (sodium *p*-aminophenylarsenate) and the proteins subsequently precipitated from the mixture with tannic acid, the precipitate invariably contained arsenic which could not be removed by washing. He further observed that when derivatives of atoxyl were used instead of atoxyl itself, arsenic was present in the precipitate only in those cases in which the derivative contained a free amino or imino group, whereas negative results were obtained with compounds such as benzoyl-acetyl-atoxyl, in which both hydrogens of the amino group were substituted, and with sodium *p*-hydroxyphenylarsenate, in which the amino group of atoxyl is replaced by hydroxyl.

In each case in which arsenic was present in the tannic acid precipitate the "atoxyl serum" could not be freed from arsenic by continued dialysis.

Breinl and Nierenstein [1908, 1909, 1 and 2] found that the same occurred when atoxyl and its derivatives were injected into animals. Arsenic was found in the serum only after the injection of compounds containing free amino or imino groups. It was concluded from these experiments that atoxyl combines with the serum proteins by means of its amino group.

The experiments described below were performed to ascertain whether similar combinations could be obtained with salvarsan and neosalvarsan. As these compounds are much less stable in solution than atoxyl, it was not possible to carry out any experiments in vitro, and only experiments with living animals were made.

For the detection of arsenic, the serum was decomposed with a mixture of sulphuric and nitric acids, all trace of nitric acid removed by continued evaporation, and the residue tested for arsenic by the modification of Gutzeit's method recommended by Sanger and Black [1907]. Care was taken to employ reagents containing no arsenic.

The Fixation of Salvarsan and Neosalvarsan by Serum.

Goats were injected intravenously with salvarsan or neosalvarsan and blood was drawn after various intervals of time. The blood was allowed to clot and the serum poured off and filtered. The serum was then dialysed in parchment bags against running water or in some cases against running saline to prevent precipitation of the globulins.

Dialysis was continued until all dialysable arsenic was eliminated from the serum, which was ascertained by dialysing for 12 hours against a litre of distilled water or saline, evaporating the dialysate to a small bulk and testing for arsenic.

The dialysed serum was then filtered and 50 cc. decomposed and tested for arsenic. In every case arsenic was found in the serum. In some cases a portion of the dialysed serum was precipitated with 2 % tannic acid solution, the precipitate was well washed and decomposed and tested for arsenic with a positive result.

These experiments show that after injections of salvarsan and neosalvarsan, some arsenic is bound to the serum proteins in a form which cannot be removed by dialysis.

A control experiment was performed in which a very dilute solution of neosalvarsan was dialysed into running water for some days and the residue, on decomposing, was found to contain no arsenic.

It was found that continued dialysis was necessary before the dialysate was obtained free from traces of arsenic, and in some cases traces dialysed out even after more than seven days. It seemed probable that the combination with the serum slowly decomposed.

(1) *Goat* 1. Injected intravenously with 0·3 g. salvarsan. After 24 hours it was bled, the serum separated and 80 cc. dialysed as above.

Serum before injection—arsenic absent.

After injection 40 cc. dialysed serum—arsenic present; 40 cc. precipitated with tannic acid—arsenic present in ppt.

(2) *Goat* 2. 0·9 g. neosalvarsan was injected intravenously and serum obtained after 7 hours and 24 hours; both dialysed as before.

Serum before injection—arsenic absent.

7 hours after injection—tannic acid ppt. from 50 cc. serum—arsenic present.

24 hours after injection 50 cc. serum used—arsenic present.

(3) *Goat* 3. 0·75 g. neosalvarsan injected intravenously, bled after 12 hours and serum dialysed.

Serum before injection 50 cc.—arsenic absent.

Serum after injection 50 cc.—arsenic present.

(4) *Goat* 4. 0·8 g. neosalvarsan injected intravenously, bled after 16 hours and serum dialysed.

Before injection 50 cc. serum—arsenic absent.

After injection 50 cc. serum—arsenic present.

An experiment was also performed to see if inorganic arsenic was bound in the serum in a similar manner. A goat was injected intravenously with an amount of arsenious acid corresponding with 0·6 g. neosalvarsan, dissolved in sodium hydrate and diluted with saline. After 24 hours blood was drawn, and the serum dialysed. 100 cc. of serum after dialysis gave no arsenic test. The same goat was subsequently injected intravenously with 0·6 g. neosalvarsan and bled after 24 hours. The serum after dialysis gave a positive arsenic reaction.

Time required for the arsenic to be completely eliminated from the blood.

Many investigations have been made on the rate of elimination of arsenic from the body after injections of salvarsan. Amongst the more recent may be mentioned that of Beveridge and Walker [1911], who found that arsenic could be detected in the urine of rabbits up to the eleventh day after intravenous injection of salvarsan and neosalvarsan. Stümpke and Siegfried [1911] claimed that traces of arsenic were found in the urine of men and other animals even after several months had elapsed, but they were unable to demonstrate the presence of arsenic in the blood later than 24 hours after intravenous injection, and they suggested that secondary depôts of arsenic were formed in the organs and then gradually excreted in the urine.

Schütte [1912] stated that with horses no arsenic could be detected in the urine and faeces later than 13 days after intravenous injection of salvarsan.

Stühmer [1914] investigated the properties of serum drawn at increasing intervals after intravenous injection of salvarsan and neosalvarsan to ascertain how long after injection it was possible to detect specific therapeutic properties in the serum. He compared the different sera with regard to their protective

action against trypanosome infection of mice, and at the same time tested the sera for the presence of free salvarsan by the Ehrlich-Bertheim reaction. The solution employed for this reaction is made by dissolving *p*-dimethyl-aminobenzene in concentrated hydrochloric acid, adding excess of mercuric chloride and dissolving any precipitate which forms by the addition of a few drops of hydrochloric acid. With this solution salvarsan gives an orange precipitate.

Stühmer found that there was a parallelism between the protective action of the sera against trypanosomes and the intensity of the Ehrlich-Bertheim reaction. Serum drawn as late as the seventh day after injection of salvarsan postponed the infection. This action was increased when the serum was heated to 56° C. and the intensity of the Ehrlich-Bertheim reaction was also increased. With neosalvarsan after the second day the serum gave no reaction either chemically or biologically. Thus after two days no free neosalvarsan appeared to be present in the serum.

Riebes [1914], employing Abelin's [1912] diazo reaction with resorcinol for detecting the amino group of salvarsan, found that in the majority of cases the reaction disappeared from serum three hours after the injection.

From these experiments it appears that free salvarsan and neosalvarsan are eliminated in a very few days from the blood.

The following experiments were made to ascertain how long a time after injection "bound arsenic" could be detected in the serum. The serum drawn at different intervals was tested for free salvarsan by the Ehrlich-Bertheim reaction, it was then dialysed into running water and the residue decomposed and tested for arsenic as before. It was found almost impossible to see the orange precipitate or coloration in the presence of the serum when very little neosalvarsan was present, the serum was therefore dialysed into water and the reagent added to the latter.

0·9 g. neosalvarsan was injected intravenously into a goat. 50 cc. serum were used in each case.

TABLE I.

Time after injection	As in dialysed serum	E.-B. reaction
7 hours	+	+
24 „	+	Faint colour
2 days	+	„
6 „	+	–
19 „	trace	–
28 „	–	–

After 19 days therefore a trace of bound arsenic was still detected in the serum, whereas a faint reaction for salvarsan was only obtained after two days.

In another experiment, Table II, 0·8 g. neosalvarsan was injected intravenously.

TABLE II.

Time after injection	As in dialysed serum	E.-B. reaction
16 hours	+	+
24 „	+	faint colour
48 „	+	–
6 days	+	–
21 „	trace	–

In this experiment a trace of arsenic was still found in the dialysed serum after twenty-one days.

From these results it is seen that the arsenic which is bound in the blood is only very slowly eliminated, whereas the bulk of the neosalvarsan is quickly excreted, only a trace being present after the first twenty-four hours.

Distribution of "bound arsenic" in the blood.

The following experiment was carried out to ascertain in what parts of the blood arsenic was retained.

A goat which had received 0·8 g. neosalvarsan was bled after 7 days so that all free neosalvarsan should be excreted. 150 cc. of blood were defibrinated, the red cells separated by centrifugalisation and repeatedly washed in the centrifuge with normal saline. The final washings from the red cells were found to be free from arsenic. The fibrin after washing was free from arsenic. Arsenic was present in the red blood cells and also in the plasma after dialysis.

Summary.

After intravenous injection of goats with salvarsan and neosalvarsan, the serum contains arsenic in a form which cannot be separated from the proteins by dialysis, and which is precipitated with the serum proteins by tannic acid. Salvarsan and neosalvarsan behave, therefore, in a similar manner to atoxyl.

No such combination is obtained when inorganic arsenic is injected.

This combined arsenic is found in the blood long after all free salvarsan and neosalvarsan have been eliminated.

This combined arsenic is found in the plasma and in the red blood cells, but no trace of arsenic is retained in the fibrin.

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XLVI. THE EFFECT OF INGESTION OF UREA, SODIUM LACTATE AND SODIUM BICARBONATE ON THE REACTION OF THE BLOOD AND THE COMPOSITION OF THE ALVEOLAR AIR IN MAN.

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The present paper records an attempt to test the question whether certain substances taken by the mouth have the effect of making the blood in the body more alkaline.

Some preliminary experiments on the subject were made by Wolf and Barcroft [1915], who in a few experiments on dogs and on Barcroft himself found that blood exposed to oxygen at 17 mm. pressure took up somewhat more of the gas after ammonium citrate or urea had been taken. This suggested increased alkalinity of the blood.

Toyojiro Kato [1915, 1] recently observed that addition of certain alkaline substances to the blood, NaOH, Na_2CO_3, $NaHCO_3$, Na_2HPO_4, accelerates the oxidation and retards the reduction of blood. Accepting this, one would expect that alkali would increase the percentage saturation of oxygen in blood exposed to a given oxygen pressure, just as acid reduces the percentage saturation [Barcroft and colleagues, 1914]. We have then used this measurement as a test for the changed reaction.

The method of exposing blood to a standard gas mixture is that described by Kato [1915, 2], in which 1–2 tenths of a cc. is put into the bulb of a small pipette, gas is then passed over it from a gas holder, the pipette being rotated in a water bath at 37°–38° C. for about ten minutes. This suffices to secure

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