sition product of ethyl nitrososemicarbazinodiacetate. However, the latter substance melted at 80° and the ureido compound from the hydrochloride of ethyl iminodiacetate at 76°. On mixing the two preparations, dissolving the mixture in chloroform, and precipitating with petrolic ether, the substance obtained melted at 80°. It would, therefore, seem that ethyl ureidodiacetate can exist in two forms, but our available supply of ethyl semicarbazinodiacetate did not admit of the preparation of sufficient of the higher melting stable form to enable a further confirmation of this view by an analysis. Both preparations were found readily soluble in the common solvents with the exception of ether and petrolic ether and both separated from chloroform solution on addition of petrolic ether in characteristic flat prisms with dome-shaped end faces. The following analysis was made on the preparation from the hydrochloride of ethyl iminodiacetate and potassium cyanate:

Calc. for $C_9H_{16}O_5N_2$: N, 12.07; found: N, 12.28.

AUSTIN, TEXAS.

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[FROM THE INSTITUTE DOYEN, PARIS.]

## A STUDY OF THE NINHYDRIN REACTION IN RELATION TO THE AGE AND HABITS OF INDIVIDUALS.

BY JOKICHI TAKAMINE, JR.

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According to Abderhalden, the blood contains a specific "Shutzferment,"[1] which lies latent in the blood but ever ready to be brought into activity when the proper conditions prevail. Such conditions are the numerous various diseases common to the human and animal kingdom. He has shown this by experiment—The ferment only being brought into mobility when a certain specific substratum was brought into contact with it and often no other.[2]

But it is not easy to conceive of the blood containing a vast number of ferments each labeled for a certain disease or group of diseases. Be that as it may, Abderhalden has shown that the blood contains a ferment only brought into activity by some special and extraneous agency. Following his method of procedure, I have determined that there is a ferment, the amount of which predominates in people of old age as compared to the amount of said ferment in young people. The amount of this ferment seems to be in direct ratio to the age of the person.

To incite the release of this ferment I have used human fibroma tissue with best results, but ordinary connective tissue will also cause similar action. I have also noted with interest that the habits of the persons whose serum was tested had a direct result upon the amount of ferment

[1] *Handbuch der Biochemischen Arbeitsmethoden*, 1912, Vol. 6, p. 223.

[2] *Z. physiol. Chem.*, 81, 90.

present or released. For example, drinkers gave tests more positive than persons of the same age with normal habits, again persons with syphilis —a disease most depletory—also gave relatively strong tests.

That this ferment is different from the "Shutzferment" is explained by its absolute absence in humans and animals (normal) of young age. Also that the "Shutzferment" cannot so greatly augment in old age—because it is at that time that we are less easily able to withstand the ravages of disease.

Therefore, for the above reasons, I have concluded that there is a ferment other than the "Shutzferment of Abderhalden" which is shown by the ninhydrin reaction to be in direct relation to the age and habits of individuals.

It is well to give here an example of one series of experiments showing the increasing amount of "ferment" with increase of age, and also the relatively great amount in the serum of a great drinker of intoxicants and a young man with syphilis.

Series No. 7.

| | | | | |
|---|---|---|---|---|
| 1 | M. B..... | 17 yrs. old | No disease | No habits |
| 2 | Mme. J..... | 27 yrs. old | No disease | Very healthy |
| 3 | M. P..... | 21 yrs. old | Syphilis | Syphilis |
| 4 | Mme. V..... | 36 yrs. old | No disease | No habits |
| 5 | Mme. I..... | 49 yrs. old | No disease | Heavy drinker |
| 6 | M. V..... | 50 yrs. old | No disease | No habits |
| 7 | Mme. N..... | 73 yrs. old | No disease | No habits |

The method[1] I will briefly state.

The serum, taken as sterile as possible, is allowed to stand in an ice chest until there is separation of the serum and clot. The former is twice centrifuged and 1.5 cc. is placed in a "Dialyser-Hülse" of Abderhalden, with about one gram of fibroma tissue. The fibroma tissue is prepared, by first washing free from blood, then boiling in distilled water. The water is changed and to the fresh water a little acetic acid is added and the solution boiled for one minute. If this water with the acetic acid gives no biuret reaction, the fibroma tissue is ready for use—placed in a sterile flask and covered with a layer of toluene. The dialyser, now containing the serum, fibroma and a little toluene is placed in a small reagent glass containing 20 cc. of distilled water and is allowed to stand at 39° for 10 hours. 10 cc. of the water solution is then boiled in a test tube with 0.2 cc. of a 1% solution of triketohydrindenhydrate for one minute.

A positive reaction means the binding of an amino group of the ferment to the ninhydrin in the X position to the carboxyl group. This combination forms a purple coloration.

[1] *Beitrage zur Klinik der Infektionskrankheiten und zur Immunitäsforschung*, 1913, p. 243.

RESULTS FROM SERIES NO. 7.

| No. | Contents of dialyser. | With ninhydrin. |
|---|---|---|
| 1 (a) | 1.5 cc. serum 1 g. fibroma tissue | — |
| 1 (b) | 1.5 cc. serum alone (control) | — |
| 2 | 1.5 cc. serum alone (control) | — |
| 3 | 1.5 cc. serum alone (control) | +++ |
| 4 | 1.5 cc. serum alone (control) | + |
| 5 | 1.5 cc. serum alone (control) | +++ |
| 6 | 1.5 cc. serum alone (control) | ++ |
| 7 | 1.5 cc. serum alone (control) | ++++ |

++++ very strong test. +++ strong test: ++ medium test:
+ weak test: — no test.

From the above series we find the following results:

1. That below the age of 27 years in healthy persons the ferment is not present or at least not sensible to tests.

2. That No. 3, aged 21, and syphilitic, gave a stronger test than a man and woman (normal) aged 36 and 49, respectively.

3. That No. 7 gave by far the strongest test, being alone due to the old age ferment.

4. That No. 6 of middle age gave a test of medium strength—he was a man of no habits.

5. That No. 5 of middle age gave an extraordinarily strong test—but she was a heavy drinker.

Experiments similar to the above were made on the serum of 68 persons with similar results.

Next it was interesting to note that the amount of the ferment was also in direct ratio to the age in animals.

An interesting series of experiments was tried with the blood of rats of different ages. Their blood serum was tested with rat sarcoma, tissue provided from the laboratories of Professors Ehrlich and Bashford, which was most active in giving a positive test with rat serum, with other albuminous matter such as human adenofibroma tissue and ordinary human connective tissue the results were generally negative. Very young rats of 85–100 grams gave no tests, rats of medium age, 115–130 grams, gave tests of medium strength, sometimes negative, and rats of old age gave strong tests.

1. The blood serum of 74 rats of 85–100 g.—always negative test.

2. The blood serum of 28 rats of 115 to 130 g.—weak positive.

3. The blood serum of 42 rats of 150–250—always strong positive test.

**Note.**—Controls of the serum alone and of the sarcoma tissue alone were always negative.

Experiments were tried on the blood of the horse and rabbit with similar results. The blood of a one month rabbit was tested in comparison to a rabbit of one year, the connective tissue of rabbits being used, with the

result that the blood of the young rabbit gave no test while that of the older gave an exceedingly strong test. Like results were obtained with the blood of a young and an old horse, the connective tissue of the horse being used.

Much speculation and many theories may be evolved to explain the significance of this ferment. But until it is isolated and its specific properties examined—work on which I am now engaged—nothing exact can be stated. Its connection with the fibrin ferment is doubtful, in that the coagulative power is greater in young and strong people than in people of older age—and since the coagulation is directly due to the fibrin ferment we see that this is in direct opposition to the action of the ferment.

Its presence may be due to the leucocytes and I have found that causing leucocytes in rabbits the blood gives stronger tests, but I have not yet determined the relative positivity of tests on young and old rabbits with leucocytes.

From my numerous experiments with the blood of human beings and animals I have found that there is a ferment in the blood shown by the ninhydrin reaction which is in direct quantitative relation to the age and habits of individuals.

Finally the test for this ferment is particularly interesting as a diagnosis of old age.

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## NEW BOOKS.

**A Laboratory Manual for the Detection of Poisons and Powerful Drugs.** By WILHELM AUTENRIETH. Authorized translation of the completely revised fourth German edition, by William H. Warren. Philadelphia: P. Blakiston's Son & Co. 320 pp., 25 illus. Price, $2.00.

This well-known book comes to us in its fourth edition, entirely revised. The subject is divided into seven chapters, and there had been considerable rearrangement of the material. After discussing poisons volatile with steam, organic poisons, and metallic poisons, the author includes in Chap. IV, a number of substances, which seldom appear in toxicological investigations, but which have much theoretical importance. Such substances as cantharidin, ergot, santonin, sulfonal, saponin substances, and the toxalbumins have become of sufficient importance to warrant a discussion here.

Special methods of analysis both qualitative and quantitative, such as methods for the determination of arsenic and antimony, salicylic acid, and special alkaloids are discussed in the next chapter. The translator has here added the Gutzeit method for the detection of arsenic and antimony, as worked out in detail by Professor Sanger. Chapter VI will prove of special interest to Pharmacists and manufacturing chemists, as it contains a very satisfactory résumé of the methods in use for the quanti-