THE MELANOPHORE REACTION AS A TEST FOR ACTH IN THE BLOOD

By

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Högberg & Johnsson (1952) and Sulman (1952) have independently published a simple test for ACTH, based on the assumption of a close relation or identity between ACTH and intermedine. Thing (1952), who is investigating the melanophore reaction for the standardization of ACTH, has recently published an extensive review on the subject in this journal. While the ascorbic acid depletion test is most time consuming and expensive, the melanophore reaction appears to be suitable as a routine hospital laboratory test. Johnsson (1952) claims to have found a positive melanophore reaction with the blood of patients with Addison's disease, Cushing's disease, myocardiac infarcts and following surgical operation (cholecystectomy). Sulman (1952) associates the pigmentation seen in Addison's and Cushing's diseases and following prolonged treatment with ACTH with the melanophore expanding hormone, stating that »the most active chromatophorotropic hormone of the hypophysis is ACTH«.

MATERIAL AND METHODS

Using the method of Johnsson as well as that of Sulman we have examined the blood of a number of patients, mainly surgical, who showed hyperfunction of the adrenals during the postoperative period as estimated from the cosinopenia and the excretion of 17-ketosteroids (Hasner et al., 1952).

Pretreatment of blood and serum.
All blood samples were placed under refrigeration immediately after withdrawal. The analyses of the samples drawn before noon were commenced the same afternoon, the samples drawn at 6 p.m. were left in the refrigerator over night.

Technique of Johnsson & Högberg: the proteins of 10 ml. of serum are precipitated
with trichloracetic acid and discarded. The supernatant fluid is acidified with sulfuric acid and the polypeptides are precipitated with phosphotungstic acid. The precipitate is extracted with barium hydroxide, the barium ion removed with sulfuric acid and the solution concentrated in vacuum to 2 ml. after the pH has been adjusted to 7. The solution is injected into 3 hypophysectomized rana esculenta or temporaria and the melanophores are read under the microscope for 2-3 hours.

Technique of Sulman: whole blood is precipitated with 0.5 per cent acetic acid in acetone. ACTH is precipitated from the supernatant fluid by further addition of acetone. The precipitate is dissolved in N/20 NaOH and neutralized. The solution is injected into light adapted hyla arborea and the macroscopic change of colour from green to brown is registered.

To check their response all animals received a trial injection of 0.0001 I. U. of ACTH («Acton») in 0.6 per cent saline. In this experiment we were able to reproduce the results of Johnsson (1952) and Sulman (1952), as in all frogs, regardless of species, the indices rose from the original 1-1.5 to 3.25-5, according to the Hogben melanophore index (Hogben, 1936). Only animals that responded with the maximal index on the test dosage were used for further experiments. A rise in index ranging up to one unit was seen in frogs given an injection of 0.5 ml. of 0.6 per cent saline. When the ACTH test solution in portions of 10 ml. (i.e., 20 times the aforementioned trial dosage) was pre-treated according to the method described by Johnsson practically all melanophore expanding activity was lost, no animal responding with an increase of index of more than one unit.

RESULTS

A patient with Addison’s disease, who was not under continuous cortisone therapy, was admitted in an acute crisis. A series of blood samples were examined before and after the institution of cortisone treatment. No significant increase or variation of melanophore expanding activity was detectable, the results ranging from 0.75 to 1.25 units.

Operation on 2 cases with Cushing’s syndrome revealed respectively diffuse cortical hyperplasia and a cortical adenoma. Neither patient showed enlargement of the sella turcica. Maximum alteration of the melanophore index was around 1 unit before as well as after surgery.

As the emphasis in this study was placed on the investigation of the post-operative reaction, the tests apart from the 3 mentioned above have been confined to patients who underwent major surgery (Table 1). The control material consisted of the same patients whose blood was tested 1 and 2 days before operation, plus a number of the personnel from the department. The melanophores were read by the author and by an assistant who was uninformed as to the results expected. The preoperative readings in 35 patients showed an
The pre- and postoperative melanophoric activities in 42 patients who underwent major surgery. The figures give the limits for the maximal activities registered (in any single frog out of the 3 used for each analysis).

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<th>Preoperative melanophoric activity</th>
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<td></td>
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<td>Hogben units</td>
<td>Colour of animals</td>
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<td>Johnsson technique</td>
<td>Patients</td>
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<td>0.75–1.25</td>
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<td>Controls</td>
<td>6</td>
<td>0.75–1.75</td>
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<td>Sulman technique</td>
<td>Patients</td>
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activity of 0.75 to 1.25 units. Six normal controls ranged between 0.75 and 1.75 units.

As there is no knowledge of the time when the blood level of ACTH is maximal it was imperative to take blood samples at short intervals during the time in which the maximum might be expected. On the other hand we know that the postoperative eosinopenia, which can be regarded as closely related to the pituitary release of ACTH, reaches its height in 4 to 6 hours (Hasner et al., 1952). Consequently blood samples have been drawn 1) at the beginning of the operation, i.e. when the anaesthesia has reached the right plane, 2) 1/2 hour after beginning of the operation, 3) 1 hour after the beginning, 4) at the close of the operation, 5) at 6 p.m. on the day of operation, 6) the morning of the first postoperative day, 7) on the second postoperative day and later on in some patients who developed severe complications. In this way we felt that we should have succeeded in registering any augmentation of the ACTH level, even if it should be of very short duration.

The patients examined underwent one of the following operations: gastric resection, total gastrectomy, resection of the colon, removal of the rectum, mastectomy with axillary and intra-thoracic dissection, nephrectomy, pyelolithotomy, suprapubic prostatectomy, and cholecystectomy.

The highest recorded variation in any single frog was 2 Hogben units, the original index of 1.25 going up to 3.25. This was observed at 6 p.m. on the day of operation in a patient who had had a resection because of a gastric ulcer. All other determinations revealed an activity of between 0.50 and 1.75 units averaging 1. No regularity or consistency can be seen in the results.

**DISCUSSION**

By systematic examination of the blood from 42 patients during the postoperative period, using the methods presented by Sulman (1952) and Johnsson (1952),
we have not been able to reproduce the results of these authors. An explanation might be found in the possibility that the release of ACTH into the blood during stress can be likened to the emptying of a depot, after which the ACTH disappears so quickly from the circulation that the process is completed between two blood-drawings. This, however, is in opposition to the findings in the investigations carried out with the Sayers' bio-assay (Bornstein & Trewella, 1950). Jores (1933) has presented evidence of a significant seasonal variation in the sensitivity of frogs with a minimum in July. Our experiments have been performed from April to October. No demonstrable variations in the results have been observed which corresponds to the results of tests with the abovementioned ACTH solution which showed no variation in sensitivity in this period, so that this possibility must also be abandoned. The objection may be raised that the method of extraction employed is unsuitable, as might be the case if the ACTH in human blood were present as ACTH protein. It might thus be discarded with the precipitated proteins as seen in the experiments already referred to, involving pretreatment of our test solution according to the method of Johnsson. Contrary to this is the work of Bornstein & Trewella (1950), who obtained positive reactions with the Sayers' test by using an extraction method which is essentially identical with that of Sulman.

Two possibilities seem to remain, first, that the amount of ACTH in the blood is too small to be detected by this method and, second, which must at present be considered the more likely, that the theory on which the method is based does not hold true, i.e. that ACTH and intermedine in their pure forms are not identical or at least do not show the close relationship assumed by Johnsson & Högberg (1952) and Sulman (1952). This agrees with reports from Geschwind, Reinhardt & Li (1952) who found a discrepancy between the activities measured by the melanophore reaction and the Sayers' test when a number of sheep ACTH preparations were assayed. The same authors also found a pronounced difference in the two activities when various parts of the pituitary gland were examined.

It is not known what is responsible for the melanophore expanding activity of some ACTH preparations. It may depend on an admixture of intermedine, but there are several other possibilities, as we know that there are many unspecific factors which produce changes in the melanophores, e.g. urethane, which when used as an anaesthetic for frogs, darkens their skin considerably, and physiological saline, which produces a weaker reaction.

**SUMMARY**

42 patients who underwent major surgery, 2 patients with hyperfunction of the adrenal cortex and one patient with Addison's disease have been examined:
no significant elevation of the ACTH level in the blood as determined by the melanophore reaction has been found. As controls the patients before the operation as well as a number of normal subjects were used. The melanophoric sensitivity of the experimental animals (frogs) was tested with a commercial ACTH preparation »Acton«. By this means a pronounced, in many even a maximal darkening of the skin was produced, which, however, seems to be caused by a substance different from ACTH.

The method, which is based on the hypothesis of identity or close relation between ACTH and intermedine, does not appear to yield reproducible results, as a consequence of which we have had to abandon it as a means of determining the stress produced during the stages of anaesthesia and operation.

REFERENCES