EXPERIMENTAL BURN STUDIES INCLUDING TREATMENT WITH CORTISONE-ACTIVE MATERIAL EXTRACTED FROM URINE*

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During the past few years improved therapy has resulted in a considerable reduction in the mortality from extensive thermal burns. This improvement has been reflected in all the clinical stages through which a severe burn passes; improvement, however, has been least satisfactory in the so-called toxic phase.

These facts are well illustrated in the data shown in Table I, abbreviated from the report of Farmer (1943), in

TABLE I.—Analysis of 1,797 Cases of Burns, Hospital for Sick Children, Toronto

Year	No. Cases	Total Mortality	Deaths				Average Day of Death*
			Shock	Toxic	Sepsis	Others	
1913-24 ..	412	35%	28%	53%	18%		1.7
1925-8 ..	230	16%	13%	40%	46%		3.4
1929-37 ..	782	11%	7%	70%	21%	2%	3.8
1938-41 ..	373	3%	0	82%	9%	9%	5.0

* 10 days and under.

which an analysis is made of 1,797 burns at the Hospital for Sick Children, Toronto, from 1913 to 1941. The table summarizes the deaths occurring within 10 days after the accident.

It will be noted that the group included under "shock" (Table I) shows the greatest improvement under modern treatment. This phase includes the clinical findings associated with a reduction in circulating blood volume, and the improvement is largely due to correction of this condition by adequate intravenous replacement therapy. Deaths from this cause are most apt to occur within the first 48 hours.

The "sepsis" group has also shown a reduction in mortality following improved local treatment and the use of chemotherapeutic agents. Included in this group are those cases which usually showed a swinging fever associated with clinical and bacteriological evidence of invasive infection of the burn-surface with or without bacteraemia or septicaemia. Death in this group is apt to be late. Under the so-called "toxic" group are included the patients who died despite initial improvement following shock treatment and who showed no obvious evidence of infection.

These changes in the problem presented by extensive burns are well reflected in the changes in the length of the survival time before fatal termination, as shown in Table I. It will be seen that the average survival time before death at present falls within the so-called toxic time-interval. There is no known effective treatment for this condition, and spontaneous recovery or death between the third and tenth days occurs despite symptomatic measures. It is here that little reduction in morbidity and mortality has been obtained, although better knowledge of fluid and electrolyte

balance and control of shock has inevitably lessened the mortality from this second phase of the burn illness. Nevertheless, it is now the greatest single factor in burn mortality.

Need for New Approaches

It thus became apparent that, no matter how excellent the shock therapy, and no matter how well the surface lesion has been treated in burns involving a considerable part of the body, the illness remains relatively unaffected by any such treatment. There is thus need for new approaches to the problem. With the recent increase in knowledge of the part played by adrenal physiology in combating stress, the striking parallelism between the physiological changes following burns and the physiological effects of A.C.T.H. and cortisone suggested that a relationship might exist between the defence response to the burn trauma and the action of these substances. The evidence for this is summarized in Table II.

TABLE II.—Comparison of the Effects of Administration of A.C.T.H., Cortisone, and the Metabolic Upset Following Burns

	A.C.T.H.	Cortisone	Burns
Nitrogen balance	Negative	Negative	Negative
17-ketosteroid excretion	Increased	Decreased followed by slight increase	Increased
Carbohydrate metabolism	Hyperglycaemia	Hyperglycaemia	Hyperglycaemia early
Sodium balance	Retention followed by excretion	Retention followed by excretion	Blood sodium drops slightly
Chloride balance	Retention followed by excretion	Retention followed by excretion	Excretion suppressed early with decrease in blood. Negative balance later
Potassium balance	Negative	Negative	Variable
Eosinophils	Reduced	Reduced	Reduced
Ascorbic acid levels	Amount in cortex reduced		Decrease in blood and excretion
Adrenal gland changes	Hypertrophy of cortex with depletion of lipid	Atrophy of cortex with depression of function	Hypertrophy of cortex with depletion of lipid

A negative nitrogen balance has been shown by Long *et al.* (1940), Ingle (1949), and Sprague *et al.* (1950) to occur after the administration of A.C.T.H. and corticosteroids of the C11 oxygenated type. It has also been shown by such authors as Cope *et al.* (1943), Levenson *et al.* (1945), and Hirshfeld *et al.* (1945) that negative nitrogen balance is a feature of the burn illness. That this nitrogen loss differs somewhat from that following other forms of stress is apparent when one considers the amount of dead tissue resulting from the burn that must be handled in some fashion. Abbott *et al.* (1946) found that the negative balance in burns was very resistant to correction by dietary means. It is interesting also to note that Sellers and You (1950) showed that deoxycortone acetate administration to burned adrenalectomized rats decreased significantly the nitrogen loss without producing marked rises in the blood N.P.N.

Sprague *et al.* (1950) reported marked increase in the 17-ketosteroid excretion in the urine after the administration of A.C.T.H. The opposite was shown to occur following cortisone administration, which suggests a depression of this cortical function when this substance is given. Cope *et al.* (1943) studied 17-ketosteroid excretion after burns and showed that there was an increased excretion for three to seven days following the burn, after which excretion dropped to normal levels or below, remaining at these levels usually until the burn was healed.

Long *et al.* (1940) demonstrated that hyperglycaemia can be induced in intact rats by the C11 oxygenated steroids when the animals are force-fed high carbohydrate diets. Changes in the glucose-tolerance curves in humans

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following the administration of A.C.T.H. or cortisone were described by Sprague *et al.* (1950). Derangement of carbohydrate metabolism, as evidenced by early hyperglycaemia, has been found after burns by such workers as Cope *et al.* (1943), Douglas (1944), and Taylor *et al.* (1944). Douglas (1944) also found that this early response was replaced later by a marked hypoglycaemia, and two of his patients required glucose therapy for hypoglycaemic reactions. Death followed in one of these cases despite temporary rises in blood sugar. This may be an indication of adrenal exhaustion.

It is noted further that there is some similarity between the electrolyte changes after the administration of A.C.T.H. and cortisone, and the alterations found following burns. However, the changes after a burn are never very marked and are usually easy to correct. Rhoads *et al.* (1941) noted increased blood chloride levels in burns following the administration of D.O.C.A., and Wilson and Stewart (1939) found that blood sodium levels rose in a similar fashion.

Of more interest is the reduction in eosinophils present in each condition. That this occurs after the administration of A.C.T.H. to humans with intact adrenals was well demonstrated by Hills *et al.* (1948). Schreiner and Pucsko (1925) first recorded this observation following burns, and noted that the disappearance of eosinophils from the blood stream was a bad prognostic sign. Reappearance was noted during the recovery phase.

Present Investigation

In our own studies we have used the method of Dunger (Thorn *et al.*, 1948), as modified by Miss C. Jackson, of the Hospital for Sick Children, Toronto, for counting the cells. This modification consists in using a solution composed of 5% eosin 5 ml., acetone 7 ml., 40% formaldehyde 3 ml., water to 100 ml.; it has been adopted because it largely removes the necessity for carrying out the count immediately upon obtaining the sample and gives better staining of the eosinophilic granules.

Our findings to date indicate a similar change to that reported by Schreiner and Pucsko. One child with a 40% third-degree burn, dying 36 hours after injury, has shown a complete disappearance of eosinophils from the blood within 24 hours. A second child with 6% second-degree burns showed a reduction to 37 cells per c.mm. A third child with 6% second-degree burns showed no striking change.

Ascorbic acid levels in the blood were shown by Abbott *et al.* (1946) to fall rapidly after burns, and Andreae and Browne (1946) recorded similar observations. On the hypothesis that the ascorbic acid was used up in the reduction of possible toxic oxidation products from the burn, thereby acting as a detoxifying agent, replacement therapy was studied by Clute, Farmer, and Franks (Brown *et al.*, 1948). We found that serum ascorbic acid levels could be raised to normal and above by either local or systemic administration of ascorbic acid. Despite such adequate levels, it was our experience that little clinical benefit was obtained during the so-called toxic phase, and, indeed, two patients died in this period with ascorbic acid levels well restored. It has been shown by Sayers and Sayers (1949) that the administration of A.C.T.H. produces a marked reduction in the ascorbic acid content of the adrenal cortex. Thus, while decreased blood levels *per se* may be of no clinical significance, they may be an index of increased A.C.T.H. activity in response to increased stress.

Sayers and Sayers (1949) have also shown that the giving of A.C.T.H. causes a marked depletion of the sudanophilic substance of the adrenal cortex. Adrenal changes following stress were shown by Greep and Deane (1949) to be most marked in the zona fasciculata and reticularis, with lesser changes in the glomerulosa. Hypertrophy of the cortex accompanies this change (Sayers and Sayers, 1949). Pursuing the burn parallel further, hypertrophy of the adrenals is a not uncommon pathological finding in fatal burns, and focal exhaustion of lipoids has been described by Douglas (1944). Hirota (1934) described early increase in thickness of the zona fasciculata and reticularis following this injury. Fig. 1 shows the adrenal gland of a child

FIG. 1.—Photomicrograph of adrenal gland of child dying on the tenth day following a severe burn. Stained with Sudan IV and counterstained. Note the loss of lipid from the zona fasciculata (F) and reticularis (R), with little change in the zona glomerulosa (G).

from our series dying on the tenth day after a 35% second-degree burn. This section, stained with Sudan IV for fat and counterstained with haematoxylin as a nuclear stain and light green for cytoplasm, illustrates well the loss of lipid from the zona fasciculata and zona reticularis, with relatively little alteration in the lipid content of the zona glomerulosa. This finding would strongly indicate that an exhausting demand has been placed on the adrenal cortex as a result of the burn injury, which proved fatal.

Experiments on Mice

If this exhaustion of cortical hormone production plays a part in the clinical course of the disease, then the administration of active cortisone (in contrast to trophic hormone—A.C.T.H.) might justifiably be considered in the therapy of severe burns. With this in mind, an experimental investigation has been carried out with thermal burns in mice to test the effect of administering cortisone-active extracts. Mice have been used because they were well suited for the planning of the experiment and the amount of active cortisone material was limited.

Adult white male mice weighing between 20 and 25 g. were used throughout. The mice were anaesthetized with intraperitoneal pentobarbitone sodium ("nembutal"), giving on the average 1 mg. of the drug. The animals were suspended, back downwards, from a balance, and counterweighted to within 65 to 75% of their body weight.

They were then immersed in water at 70° C. to a depth sufficient to restore balance, and held there for 15 seconds. The mice were thus burned over a surface covering 30% of their body weight. This method gave a constant fractional burn for each animal.

Since it has been shown that the administration of plasma can largely eliminate shock as a cause of death, all the treated animals were given intravenously a solution of pooled human serum albumin in distilled water as soon as possible after the burn. The material was diluted to form a 12.5 or 6.25% solution, and the mice received on the average 0.5 ml. of the albumin solution over a period of one hour. This is equivalent in amount to about 25% of their blood volume. In this fashion we have attempted to study the conditions obtained in a severely burned patient in whom shock has been controlled, thus simulating the current clinical problem with the exception that no attempt was made to treat the lesion locally. Follow-up of the animals showed that this method produced mixed second- and third-degree burns of approximately 30% surface area. By way of control on the intravenous therapy alone, a further group of animals was left without any treatment whatever following the trauma.

Cortisone-active material was obtained from pooled human adult male day urine, extracted by a modification of the method used by Venning *et al.* (1946) for the bio-assay of glycosidic corticoids. For administration to the animals the dried extract obtained routinely from four-litre samples of urine was taken up in 0.3 ml. of absolute alcohol, to which were added 1.5 ml. of sterile 10% glucose (obtained by filtering through a Seitz filter) and 1.2 ml. of sterile distilled water. This gave 3 ml. of a variable light-brown emulsion for each four-litre urine residue.

To test the effect, half of the animals receiving the intravenous therapy were injected twice daily, subcutaneously, with 0.5 ml. of this emulsion in an area remote from the burn. Venning and Browne (1949) demonstrated that the average human male 24-hour excretion of urine contained approximately 60 glycosidic units of corticosteroids—one glycosidic unit being defined as the amount of material causing a deposit of liver glycogen similar to that deposited by 1 μ g. of cortisone (compound E of Kendall). Computing from these figures of Venning, it follows that each burned animal received about 40 glycosidic units of cortisone-equivalent material daily. Treatment was carried on for four days after the burn. This dosage and time was chosen as representing a known biologically active amount of hormone for the mouse, given over a period when the so-called toxic effects were judged to be most likely to occur. Likewise, the intravenously treated control mice were given twice-daily injections of 0.5 ml. of an aqueous solution of 5% glucose and 10% alcohol over a similar period.

Results

The results of the study of 41 untreated control mice and 69 animals treated with intravenous shock therapy alone, as compared with the results from 69 mice given the above cortisone treatment plus intravenous shock therapy, are shown in Fig. 2. It will be seen that the administration of cortisone-active material has markedly reduced the burn mortality in the mice which, in addition, receive intravenous therapy for shock. This reduction is pronounced even in comparison with the group receiving the shock therapy alone. The probabilities of the significance of the difference in death rates found between the latter two groups are plotted against days after the burn, as shown in Fig. 3. It will be seen that a significant dif-

ference ($P < 0.05$) is established in the findings throughout the experiment. The improvement in death rate becomes highly significant ($P < 0.02$) between one and seven days after the burn despite the lack of local treatment of the lesion.

It will be noted also from Fig. 2 that, as was to be expected, the intravenous therapy alone has reduced the

FIG. 2.—Effect of intravenous shock treatment and of cortisone-active extract on mortality following thermal burns in mice.

mortality considerably as compared with the untreated controls. In addition the graph shows that the shock therapy resulted in a longer period of survival following the burn in those animals which eventually succumbed. These experimental findings with the mice parallel the clinical results following shock therapy as shown in Table I.

FIG. 3.—P of the difference plotted against time after burn of cortisone-plus-shock-treated mice as compared with shock treatment alone.

The experimental study thus accurately reflects the clinical experience in these respects. An examination of the actual times of death following the experimental burn shows that, of those animals dying under seven days, the average survival time of the lethal cases for the untreated controls was eight hours; for the shock-treated group it was 12 hours, and for the cortisone-plus-shock-treated it

was 19 hours. It will be seen that this interval shows a lengthening as a result of the shock therapy, yet this increased survival time was increased still further as a result of the cortisone-active material. It should be noted that among the seven deaths occurring before seven days with the cortisone-treated group are included two animals which died within ten minutes of the burn, before intravenous therapy was completed. If these are omitted the results become even more striking.

The locally untreated burn lesions went on to sloughing and ulceration. It is considered that the deaths after seven days following the burn largely resulted from the untreated lesion and therefore do not constitute part of the present study.

That the cortisone effect was exerted on the toxic phase rather than on the shock period cannot be dogmatically stated, yet it is significant to note that after the intravenous therapy the animals appeared relatively normal for a while, and would later become inactive and die. The further reduction in the death rate of the shock-treated animals resulting from the additional treatment with the cortisone-active material, coupled with a further increase in the survival time of the lethal cases in this group, may be interpreted as evidence beyond what might possibly be expected if the effect was on the shock mechanism alone. In this connexion the reputed antihistaminic action of cortisone may be of interest.

There is thus evidence that the administration of urinary extracts known to contain cortisone-active fractions has a beneficial effect in reducing mortality of experimentally burned mice. This would seem to indicate that it would be justifiable to give cortisone to clinical burns from which death might be anticipated during the so-called toxic phase.

In the present study, evidence of thinning of the epidermis and atrophy of sebaceous glands, as stated by Li (1950) to occur with A.C.T.H. administration, was not sought by microscopical examination. However, the purple striae and thinning of the epidermis noted by Sprague *et al.* (1950) with excess cortisone administration were not found in the cortisone-plus-shock-treated animals; nor was there any gross evidence that this treated group developed proportionately greater areas of third-degree burn or showed delayed or imperfect healing as reported by Ragan *et al.* (1950) following excess cortisone administration. These points must, however, be considered in the clinical application of cortisone to burned patients. It is probable that, clinically, the aim should be to restore the cortisone level to an amount adequate for the body to meet the emergency, thus supplementing the output from an exhausted adrenal gland, rather than to produce an excess of the hormone which in itself might have adverse effects.

It would at first glance seem to be contradictory to give a burned patient a substance which will produce many of the metabolic changes that a burned individual already shows. If, however, these findings are viewed from the standpoint that the changes result from the patient's own defence-response to the insult, which may be exhausted in the process, the therapy becomes logical. The administration of A.C.T.H., on the other hand, might not be expected to be useful if the adrenal gland is already exhausted.

Consideration might well be given to the possibility of the withdrawal effects, in view of the need for such surgical measures as skin-grafting within the week following treatment. If adrenal cortical activity is depressed after the administration of an excess of cortisone it might be undesirable to submit the patient to an operative stress while this state of affairs was present.

It is also apparent that, by carrying out the rather simple extractions of urine, as used in the methods for bio-assay of glycolytic corticosteroids, amounts of cortisone-active extract can be obtained sufficient for experimental work, which can be carried out with small animals.

Summary

Further evidence is presented that clinically there may be an adrenal exhaustion in severe burns.

Deaths from experimental thermal burns can be markedly reduced by treatment with a urinary extract containing cortisone-active material combined with intravenous shock therapy.

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REFERENCES

- Abbott, W. E., Hirshfeld, J. W., Williams, H. H., Pilling, M. A., and Meyer, F. L. (1946). *Surgery*, **20**, 284.
 Andrae, W. A., and Browne, J. S. L. (1946). *Canad. med. Ass. J.*, **55**, 425.
 Brown, W. A., Farmer, A. W., and Franks, W. R. (1948). *Amer. J. Surg.*, **76**, 594.
 Cope, O., Nathanson, I. T., Rourke, G. M., and Wilson, H. (1943). *Ann. Surg.*, **117**, 937.
 Douglas, B. (1944). *Surgery*, **15**, 96.
 Farmer, A. W. (1943). *Amer. J. Surg.*, **59**, 195.
 Greep, R. O., and Deane, H. W. (1949). *Ann. N.Y. Acad. Sci.*, **50**, 596.
 Hills, A. G., Forsham, P. H., and Finch, C. A. (1948). *Blood*, **3**, 755.
 Hirota, K. (1934). *Nagasaki Igakkwai Zasshi*, **12**, 1143. Quoted by H. N. Harkins, *The Treatment of Burns*, 1942. Thomas, Springfield, Ill.
 Hirshfeld, J. W., Abbott, W. E., Pilling, M. A., Heller, C. G., Meyer, F., Williams, H. H., Richards, A. J., and Obi, R. (1945). *Arch. Surg., Chicago*, **50**, 194.
 Ingle, D. J. (1949). *Ann. N.Y. Acad. Sci.*, **50**, 576.
 Levenson, S. M., Davidson, C. S., Lund, C. C., and Taylor, F. H. L. (1945). *Surg. Gynec. Obstet.*, **80**, 449.
 Li, C. H. (1950). *British Medical Journal*, **1**, 299.
 Long, C. N. H., Katzin, B., and Fry, E. G. (1940). *Endocrinology*, **26**, 309.
 Ragan, C., Howes, E. L., Plotz, C. M., Meyer, K., Blunt, J. W., and Lattes, R. (1950). *Bull. N.Y. Acad. Med.*, **26**, 251.
 Rhoads, J. E., Wolff, W. A., and Lee, W. E. (1941). *Ann. Surg.*, **11**, 955.
 Sayers, G., and Sayers, M. A. (1949). *Ann. N.Y. Acad. Sci.*, **50**, 522.
 Schreiner, K., and Pucsko, O. (1925). *Med. Klinik.*, **21**, 1882, 1925.
 Sellers, E. A., and You, S. A. (1950). Unpublished Report.
 Sprague, R. G., Power, M. H., Mason, H. L., Albert, A., Mathieson, D. R., Hench, P. S., Kendall, E. C., Slocumb, C. H., and Polley, H. F. (1950). *Arch. intern. Med.*, **85**, 199.
 Taylor, F. H. L., Levenson, S. M., and Adams, M. A. (1944). *New Engl. J. Med.*, **231**, 437.
 Thorn, G. W., Forsham, P. H., Prunty, F. T. G., and Hills, A. G. (1948). *J. Amer. med. Ass.*, **137**, 1005.
 Venning, E. H., and Browne, J. S. L. (1949). *Ann. N.Y. Acad. Sci.*, **50**, 627.
 — Kazmin, V. E., and Bell, J. C. (1946). *Endocrinology*, **38**, 79.
 Wilson, W. C., and Stewart, C. P. (1939). *Edinb. med. J.*, **46**, Trans. Med.-Chir. Soc., p. 153.

Meetings of the International Optical Commission were held in London at the Royal Society on July 17 and 18. The commission, which is part of the International Union of Pure and Applied Physics, was constituted at Delft in 1948 after a preparatory meeting in Prague in 1947; its purpose is to further international scientific relations in the field of optics. The London meeting is the first since the formation of the commission, and there were some 30 representatives from Great Britain, Belgium, Spain, the U.S.A., France, Italy, the Netherlands, Poland, Sweden, Switzerland, and Czechoslovakia. The president of the commission is Mr. T. Smith, F.R.S. (Great Britain), and the secretary Professor P. Fleury, of the Institut d'Optique, in Paris. The commission held joint meetings with the London Conference on Optical Instruments at the Imperial College of Science and Technology in South Kensington on July 19–26.