ON THE MECHANISM THROUGH WHICH OBSTRUCTIVE JAUNDICE INFLUENCES INFLAMMATORY PROCESSES*

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(RECEIVED FOR PUBLICATION MARCH 3, 1954)

A variety of experiments on animals had shown that the antiphlogistic effect of systemic stress is largely non-specific, in that it can be elicited by various agents and is effective in suppressing inflammation in various sites. For instance, systemic stress induced by diverse procedures, if it is sufficiently severe to elicit an intense general adaptation syndrome, markedly inhibits the so-called “histamine appendicitis” (Selye, 1936, 1937a), different varieties of acute inflammatory lung-oedema (Selye, 1938a, 1938b), and the anaphylactoid inflammation caused by egg-white in the rat (Selye, 1937b; Léger, 1948). Subsequently, it became evident that—at least under certain “conditioning” circumstances—systemic stress can also facilitate the production of inflammatory lesions (e.g. nephritis and myocarditis after exposure to cold). The adrenals were suspected of playing an important part both in the inhibition and in the enhancement of inflammatory phenomena by stress, since they were found to be greatly enlarged during the general adaptation syndrome, and since both the anti- and the prophlogistic effects of stress could be prevented by adrenalectomy (Selye, 1937b, 1946).

When pure corticoids became available, it was noted that some, the “mineralo-corticoids”, are also “prophlogistic corticoids” (e.g. desoxycorticosterone) in that they stimulate, while others, the “gluco-corticoids”, are also “antiphlogistic corticoids” (e.g. cortisol or cortisone) in that they inhibit inflammation (Selye, 1949; Selye and Pentz, 1943). However, the effects of endogenous cortical hormones upon inflammation are not solely dependent upon the rate of their production; the activity of these corticoids can be considerably modified by a variety of “conditioning factors” (Engel, 1953; Selye, 1954). The liver had long been suspected of playing an important role in this connexion. In the course of studies on the mechanism of this conditioning, we came to examine the influence of various experimentally induced hepatic injuries (partial hepatectomy, ligature of the common bile-duct) upon the actions of desoxycorticosterone, the first corticoid to be made available by synthesis (Selye, 1941, 1943; Selye and Stone, 1944). It was found that the effectiveness of this and many allied steroids can be strikingly enhanced by partial removal of hepatic tissue.

Independently, clinical experience had shown that, in patients with hepatic damage, inflammatory processes, and, in particular, rheumatoid lesions, tend to regress. The question arose whether this beneficial effect is merely due to an increased endogenous production of ACTH and of antiphlogistic corticoids—due to the systemic stress caused by the hepatic damage—or whether the liver is also more specifically involved in conditioning the efficacy of the corticoids after they are discharged into the blood. We were especially interested in clarifying this point, because of its practical importance. At present one of the greatest handicaps in the clinical use of antiphlogistic corticoids (e.g. for the treatment of rheumatoid and allied inflammatory conditions) is that often they are effective only at comparatively high dose-levels, at which unpleasant side-effects are rather common. A better understanding of the mechanism through which inflamed tissue can be “conditioned” or sensitized to antiphlogistic corticoids might show us how to obtain optimal effects with relatively low and safe doses (Selye, 1952; Selye and Horava, 1953).

The purpose of this communication is to report upon experiments in which the common bile-duct was severed in adrenalectomized rats which were maintained either with an antiphlogistic (cortisone) or a prophlogistic (desoxycorticosterone) hormone. A standardized, objectively-measurable inflammatory focus was then produced as an indicator of

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* This work was supported (in part) by the Medical Research Board, Office of the Surgeon-General, Department of the Army, Contract No. DA-49-007-Md 186, and by a Consolidated Grant from the National Research Council of Canada.
the animals’ phlogistic potential. Here, the obstruction of the bile-duct could not have altered the secretion of corticoids, since the animals had no adrenals, but it could still influence the efficacy of the corticoids administered to them at an unvaried daily dose-level.

Experimental Materials and Techniques

Experimental Animals and their Maintenance.—Six groups of animals, each consisting of ten female Sprague-Dawley rats, were used. Their treatment as well as their initial and final body weights are listed, together with our findings, in the Table. All animals were fed on “Purina Fox Chow” and, since fifty of them were adrenalec-tomized and in need of sodium supplements, for uniformity’s sake, all received 1 per cent. NaCl as drinking fluid.

“Granuloma-Pouch” Technique.—For the production and quantitative assessment of a long-lasting inflammation, the “granuloma-pouch” technique was used. The details of this procedure have been described elsewhere (Selye, 1953a, 1953b), but it should be stated here that the principle of the procedure is to inject 25 ml. air under the shaved dorsal skin of the rat, this being immediately followed by the introduction of some irritant into the regularly shaped, ellipsoid connective-tissue chamber thus formed. In the present experimental series, 0·5 ml. of 0·5 per cent. croton-oil solution (in corn oil) was used as a topical stressor to stimulate inflammation. The resulting amount of haemorrhagic inflammatory exudate can be assessed approximately, day-by-day, by transillumination of the pouch with an electric flashlight; it can subsequently be measured accurately by withdrawing the fluid into a graduated syringe. This measurement served as an objective indicator of inflammation and was performed after the animals were killed, on the 12th day following the preparation of the pouch. The granulomatous wall itself was not weighed in this experimental series, but macroscopic inspection and the assessment of its width on histological sections showed that there is a close parallelism between the volume of the exudate and the thickness of the granulomatous pouch.

Topical Irritation Arthritis.—On the 12th day, 2 hrs before the animals were killed, a so-called topical irritation arthritis test was performed in all groups. This enabled us to compare the behaviour of the more chronic exudative type of inflammation in the pouch, with that of an acute “anaphylactoid” reaction. The latter can be obtained by injecting certain agents to which the rat is hypersensitive (e.g., egg-white, dextran, globin, etc.) into the subcutaneous tissue of the paw. The significance of this “anaphylactoid inflammation” has been described in several earlier publications (Selye, 1937b; Jasmin and Robert, 1953) and need not be discussed here. In the present experiment, 0·2 ml. of 0·2 per cent. dextran solution (prepared by diluting the commercial 6 per cent. dextran 1 : 30 with water) was injected under the plantar skin of the left hind-paw of each rat. The anaphylactoid inflammatory oedema was then objectively assessed by comparing the weights of both hind paws after amputation through the ankle joint. If the paw is amputated without previous fixation, much of the inflammatory fluid is lost. Hence, immediately after death, the hind limb was resected at the knee-joint and fixed in Bouin’s solution; 24 hrs later the oedematous inflamed tissue became hardened and the paw could be cut off, with a sharp incision through the tibio-tarsal joint, without loss of fluid.

Adrenalectomy.—All adrenalectomies were performed under ether anaesthesia through the lumbar approach, 48 hrs before the preparation of the granuloma-pouch. This interval permitted the elimination of circulating corticoids and recovery from the operation.

Corticoid Substitution Therapy.—In Groups II and III, 1 mg. cortisone acetate microcrystals in 0·2 ml. suspending agent was given subcutaneously daily: in Groups V and VI, desoxycorticosterone acetate (DCA) microcrystals were administered at the same dose-level and in the same manner.

Bile-Duct Ligature.—The ligature of the common bile-duct was performed in Groups IV, V, and VI, under ether anaesthesia, immediately after the granuloma-pouch was prepared. A mid-line incision was made in the epigastric region through the shaved abdominal wall, and the duodenum was exteriorized. This exposed the bile-duct which was freed from adjacent pancreatic tissue just above its entrance into the gut, and transcised between two ligatures. After this, the abdominal wall was closed with three stitches.

Results

On the 12th day after the preparation of the granuloma-pouch, all animals were killed with chloroform, the exudate was measured as outlined above, and the thymus, spleen, preputial glands, both hind-paws, and a specimen of the granuloma-pouch wall, were fixed in Bouin’s solution for histological study. All these organs—except the segment of granuloma-pouch—were also weighed (Table, overleaf).

Body-Weight.—It will be noted that there was no significant loss in any of the groups, but the normal growth was suppressed by the bile-duct ligature in the intact (Group IV) and the adrenalectomized-cortisone-treated (Group V) rats. The final body weight of both DCA-treated groups was comparatively high and almost the same, whether they suffered from jaundice (Group VI) or not (Group III). This may have been due partly to imperceptible oedema-formation, under the influence of the sodium-retaining corticoid. However, among the jaundiced animals, the absence of body-weight loss was undoubtedly also conditioned by the fact that catabolism during jaundice (as in many other conditions of stress) is largely dependent upon the availability of gluco-corticoids.
Inflammatory Exudate.—The formation of exudate was markedly, but incompletely, suppressed by bile-duct ligature in the intact rats (Group IV). On the other hand, not a single adrenalectomized rat maintained on cortisone showed the slightest trace of exudate formation after ligature of the bile-duct (Group V). As a matter of fact, even the connective tissue lining the granuloma-pouch showed no evidence of any response to the direct effect of croton oil. Conversely, in the DCA-treated-adrenalectomized rats (Group VI), the ligature of the bile-duct failed to suppress inflammatory exudate formation. The apparent difference between the volume of exudate in this group and in the adrenalectomized-DCA-treated controls, in which the bile-duct was not ligated (Group III), was statistically not significant (the value of “P” being between 0·4 and 0·5). In other words, under our experimental conditions, jaundice diminished inflammatory exudate formation in the presence of the adrenals; after adrenalectomy this effect was accentuated in animals maintained on cortisone and counteracted in those maintained on DCA. Thus there was a true synergism between the effects of cortisone and of jaundice in the adrenalectomized rat. This view is substantiated by the observation that the same dose of cortisone, given to adrenalectomized animals with intact bile-ducts (Group II), caused only a moderate depression of exudation, as compared to the untreated controls (Group I). These findings are perhaps even more striking when we contemplate dissected preparations of the granuloma-pouches, as represented in Fig. 1 (opposite).

Thymus.—Involution is an excellent indicator of the lympholytic effect of antiphlogistic corticoids, and this action roughly parallels their anti-inflammatory potency. It is noteworthy, therefore, that, in adrenalectomized controls (Group II), the dose of cortisone used in these experiments resulted only in a slight depression of the thymus-weight below that of the intact untreated controls (Group I). The thymus of the adrenalectomized-DCA-treated rats (Group III) was actually larger than that of the intact controls. This confirms that DCA, a prophylogistic corticoid, has no thymolytic effect. It should not be considered as a proof of an actual thymotrophic action, however, since adrenalectomy in itself notoriously results in some thymus enlargement, due to the elimination of all endogenous antiphlogistic (cortisone-like) hormones. It is also noteworthy that in intact rats (Group IV), the thymolytic effect of jaundice was much less pronounced than in adrenalectomized-cortisone-treated animals (Group V). In the latter, virtually the entire parenchyma of the organ had disappeared and only the stromal structures—which usually weigh about 40-60 mg.—were left. Thus, here again, there is a true synergism between jaundice and cortisone. This could not have been due merely to an increased secretion of cortisone-like compounds under the influence of jaundice, since these rats were adrenalectomized. No such synergism was noted between jaundice and DCA in adrenalectomized animals (Group VI), and it is even doubtful whether, under these conditions, the ligature of the bile-duct had any effect upon thymus weight. It is true that here, the mean weight of the organ is slightly below that of adrenalectomized-DCA-treated controls (Group III),
but statistically, the difference is not significant (the value of "P" being between 0.1 and 0.2).

Spleen.—This is only partly lymphoid, and usually tends to respond less markedly but in the same manner as the thymus in animals treated with cortisone or exposed to stress. This is essentially confirmed by the present experimental series. We noted only an insignificant decrease of splenic weight in intact jaundiced animals (Group IV); this involution was greater (though not significantly so) in the adrenalectomized-corticosterone-treated rats (Group V), and was completely absent in adrenalectomized-DCA-treated rats (Group VI). In fact, in the latter group, the weight of the spleen was the same (the apparent increase is not statistically significant) as in the adrenalectomized-DCA-treated controls without bile-duct ligature (Group III).

Preputial Glands.—Considerable importance is attached to the response of these glands, for reasons which will be explained in the discussion. Meanwhile, let us merely point out that, irrespective of all other variables, in the three groups of jaundiced animals, the weights of these glands were actually somewhat below normal. In comparing any one jaundiced group with the corresponding non-jaundiced group, this atrophy is of doubtful statistical significance. However, its constancy in all three groups with bile-duct ligature is highly suggestive and, in any event, for our interpretation, the important fact is the absence of a preputial-gland hypertrophy.

Topical Irritation Arthritis Test.—This was evaluated on the basis of the difference between the right (control) and left (dextran-injected) hind paws. Under the circumstances of this experiment, DCA produced no significant change in any of the groups, while cortisone induced a noteworthy inhibition of inflammatory swelling only in the adrenalectomized-jaundiced rats (Group V). That this particularly acute type of inflammation is more difficult to influence with hormones than the more chronic process in the granuloma-pouch, agrees with the observations of most workers in this field, including our own. It is noteworthy, however, that even here, a definite sensitization to cortisone could be induced in adrenalectomized animals by the ligature of the bile-duct.
Discussion

From these experiments, it is evident that in intact rats, ligation of the common bile-duct affects inflammation and the lymphatic organs in the same manner as a heavy overdosage with antiphlogistic corticoids. In particular, it inhibits inflammatory exudation and causes involution of the thymus.

In analysing the mechanism through which these effects might take place, the following questions arise:

(1) Are the antiphlogistic and thymolytic effects of bile-duct ligation merely due to the non-specific stress of this surgical intervention, or to the resulting retention of bile?

In order to examine this point, a subsidiary experiment was performed on ten intact rats, comparable in every respect to those of Group IV, except that the bile-duct was not ligated but merely exposed and freed of surrounding pancreatic tissue. Here, the mean volume of exudate accumulated during 12 days in the granuloma-pouch (8.9 ± 0.89) and the weight of the thymus (218 ± 9.5) were essentially the same as in the intact controls (Group I) of the present experimental series. It may therefore be concluded that the stress of the surgical operation itself was not the decisive factor. It still remains to be seen whether the jaundice acted specifically through some components of the retained bile, or through damage to the liver, caused by the mere pressure of its accumulating secretion.

(2) Is the synergism between bile-duct ligation and cortisone a mere summation or an actual potentiation of their individual actions?

We doubt whether this question can be definitely answered on the basis of our experiments, but consider the latter possibility to be more probable. It will be recalled that the effect of bile-duct ligation upon exudation and thymolysis was not statistically significant in DCA-treated-adrenalectomized animals; yet, in cortisone-treated-adrenalectomized rats, the experimental jaundice caused complete inhibition of exudation and virtually total involution of the thymus. A mere summation of effects is rendered even less likely when we consider the topical irritation arthritis as an indicator of inflammation and the changes in the splenic weight as a sign of lympholysis. In adrenalectomized animals, a definite inhibition of the arthritis with splenic atrophy was observed only in jaundiced rats treated with cortisone. As judged by the lack of response of adrenalectomized-DCA-treated rats, jaundice exerts no direct effect upon the arthritis or upon splenic weight. In any event, however,—be this summation or potentiation—it is clear that even after adrenalectomy jaundice “conditions” inflamed and lymphatic tissue to the inhibitory effect of cortisone and hence that it must act through some extra-adrenal mechanism.

(3) Could this conditioning effect of jaundice be mediated through the discharge of endogenous ACTH?

The channels through which various systemic stressor agents condition the body to the actions of antiphlogistic corticoids, such as cortisone, have been reviewed at some length in another publication (Selye, 1954). These possible pathways are schematically outlined in Fig. 2.

The topical response (e.g., inflammation, cell-degeneration, and necrosis) to a local stressor, in our case croton oil, can be influenced by a systemic stressor, in our case jaundice, through a dual mechanism:

(I) The systemic stressor induces an ACTH-discharge by the pituitary; this leads to an increased secretion of antiphlogistic corticoids (A-C) of the cortisone-type. The latter inhibit inflammatory phenomena by virtue of their direct effect upon mesenchymal tissues (represented here by a fibroblast).

(II) This effect is greatly enhanced by some non-adrenal-mediated “antiphlogistic-corticoid-conditioning-factor(s)” (A-CC). It has not yet been established whether this A-CC is related to the “first mediator” (designated here by a question mark), which is responsible for the ACTH-discharge during stress. There is some evidence, however, that ACTH itself may—in addition to its trans-adrenal action—possess a peripheral A-CC effect (dotted line).

Experimental evidence, suggesting the existence of such a non-adrenal-mediated direct action of ACTH, has been presented in several previous publications (Jacot and Selye, 1952; Selye, 1951; Selye and Jacot, 1952). In particular, it had been shown that various impure ACTH preparations cause involution of the thymus in adrenalectomized rats maintained

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**Fig. 2.**—Pathways through which a systemic stressor agent (e.g., jaundice) could affect inflammatory responses to local stress (e.g., that caused by a chemical irritant such as croton oil).
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on very small doses of cortisone, which, in themselves, are inactive in this respect. However, this thymolytic effect was not obtained with more purified ACTH preparations; hence, the possibility had to be considered that the thymolytic factor, present in impure preparations, is either a separate hormone, or merely the manifestation of systemic stress caused by impurities. The latter possibility is rendered rather probable by other observations of this laboratory (Selye, 1950; Herlant, 1950). It was noted that in adrenalectomized rats—maintained exclusively with salt supplements to their diet—there is no thymolysis during systemic stress, but that marked thymolysis occurs if they are maintained with small doses of cortisone, which in themselves are ineffective.

Another extra-adrenal action of ACTH-containing extracts is their ability to stimulate the preputial glands of the rat. Unlike the thymolytic action, the effect upon the preputial glands was obtained even with the most purified preparations of ACTH (Jacot and Selye, 1952; Selye and Jacot, 1952). In recent (hitherto unpublished) experiments we confirmed this also with the highly purified "Corticotropin-B".* It cannot be excluded that even the best available ACTH preparations are contaminated with a separate trophic hormone for the preputial glands, but we have no factual evidence for this assumption. It is of special interest, therefore, that jaundice caused no hypertrophy of the preputial glands in any of our rats. Unless the preputial-gland-stimulating effect of ACTH preparations is due to a contamination, it may be concluded, therefore, that the A-CC action of jaundice is not mediated by a direct effect of endogenous ACTH (as indicated by the dotted line in our drawing). This is all the more noteworthy, since Hess and others (1952) claimed that the systemic stress caused by burns is associated with a stimulation of the preputial glands which they ascribed to the discharge of endogenous ACTH. It is undoubtedly true, furthermore, that jaundice does cause an adreno-cortical enlargement, which is presumably mediated through the discharge of ACTH; hence this aspect of our findings cannot be adequately explained at present. There is some reason to suspect the existence of several kinds of ACTH. It is possible that the corticotrophic principle secreted during jaundice is insufficient in amount or that it is devoid of any preputial-gland-stimulating action. Furthermore, this particular effect of the hormone may be suppressed by some other action of jaundice. Be this as it may, the observations described in this communication offer no support for the assumption that the A-CC effect of jaundice is mediated through some extra-adrenal action of ACTH.

In conclusion, it may be said that our experiments furnish definite evidence in favour of the view that the well-known anti-inflammatory effect of jaundice is not merely due to a direct action upon connective tissue, nor exclusively mediated through the stimulation of antiphlogistic-hormone secretion. It depends, at least to a considerable extent, upon a peripheral synergism between antiphlogistic corticoids and some extra-adrenal "conditioning" effect of the jaundice. Although our diagram deals only with inflammatory changes, the same factors appear to be involved in the lympholysis caused by jaundice, except that here no topical stressor is applied to the target-organ directly.

Summary

Jaundice, experimentally induced by ligature of the common bile-duct, inhibits inflammation in the rat. This can be assessed in quantitative terms by the measurement of exudation in the "granuloma-pouch" test. At the same time, jaundice also causes pronounced involution of the thymus.

These effects of jaundice are not merely the result of an increased antiphlogistic-corticoid production, since they are evident even in adrenalectomized animals, maintained on small doses of cortisone (in themselves virtually ineffective).

In adrenalectomized rats, maintained with desoxy-corticosterone (which is devoid of antiphlogistic actions), this effect of jaundice is either absent, or very slight.

Jaundice (like most other agents which cause intense systemic stress) increases the secretion of ACTH, and consequently of antiphlogistic corticoids; yet it is not primarily through this mechanism, nor through a direct peripheral effect of the bile, that it inhibits inflammation or causes thymolysis.

The experiments suggest that the intense inhibition of inflammatory and lymphatic tissues by jaundice is primarily due to a conditioning or sensitization of the tissues to antiphlogistic corticoids, the so-called "A-CC effect".

The author is greatly indebted to Doctor Ernesto Salgado for all statistical calculations, and to Mr. Kai Nielsen, Miss M. Langlois, and Miss R. Prud'homme for technical assistance, including the preparation of the illustrations.

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Sur le mécanisme par lequel l'ictère par rétention influence le processus inflammatoire

RÉSUMÉ

Un ictère reproduit expérimentalement par la ligature du canal bilaire inhibe l'inflammation chez le rat. Ceci peut être détermine quantitativement par la mesure de l'exsusdat dans la "poche granulomateuse". En même temps l'ictère produit l'involution du thymus.

Ces effets de l'ictère ne derivent pas simplement de la production corticoide antiphlogistique augmentee, puis qu'ils se voient aussi chez des animaux adrenalectomises et maintenus par de petites doses de cortisone (étant seules virtuellement sans effet).

Chez des rats adrenalectomises maintenus par la desoxycorticosterone (qui n'a pas d'action antiphlogistique) cet effet de l'ictère est léger ou absent.

L'ictère (comme tout agent qui produit un état d'intense fatigue générale) augmente la sécrétion de l'ACTH et par conséquent des corticoïdes antiphlogistiques; toutefois, ce n'est pas en premier lieu par ce mécanisme ni par un effet périphérique direct de la bile qu'il inhibe l'inflammation ou produit la thymolyse.

Les expériences suggèrent que l'inhibition intense des tissus inflammatoires et lymphatiques par l'ictère est due en premier lieu au conditionnement et à la sensibilisation des tissus aux corticoïdes antiphlogistiques, autrement dit à "l'effet A-CC".
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doi: 10.1136/ard.13.2.102

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