rise to increased bilirubin production reported here, the block in reutilization of iron must occur at some point after the release of iron from heme since catabolism of the porphyrin moiety proceeded normally.

Summary. Bilirubin production was found to increase twofold during the first 24 hr after administering 100 μg endotoxin (intraperitoneally) to rats. A 50% increase in bilirubin production was observed in host rats 7 days after transplant of the Walker carcinoma 256 (intramuscularly). Ability of the liver to conjugate bilirubin was normal in both types of animals. The results obtained are consistent with the view that the concomitant block in re-utilization of iron occurs at some point after its release from heme.

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Proliferation and Resistance of Epidermis in Response to Harmful Stimuli* (33376)

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Proliferation and hyperplasia of cells are phenomena observed widely in various biologic systems. In such effects of overgrowth, it is obvious that the dimensions of the involved tissue exceed those established by normal controls on growth. Very little, however, is understood about this, even as to initiating circumstances (1–3).

One phenomenon that might be explored for possible significance in proliferative overgrowth is the biologic reaction of repair, that is, regeneration after loss of part of a given tissue. In other words, it may be asked

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whether hyperplasia could be a quite regular result of the regenerative repair response, a result brought out even more when the damage is chronic or repeated.

More specifically, it may be asked whether one or more adequately harmful stimuli to a group of cells would be followed regularly by a rise above normal in the quantity and size of the cells and subsequently by a return to normal. It was felt that the answer to this was desirable first in simple histologic terms as preparation for a biochemical exploration of the mechanisms and effects of regenerative response to damage. Histologic evidence presented here indicates that any of a large number of harmful substances regularly produce a proliferative response in the skin of mouse and guinea pig. Various characteristics of the effect are reported, together with certain related biologic principles.

The constancy of a hyperplastic response to harmful substances reported here raises a second, early question: Is the repletive-proliferation response, after application of a harmful agent, associated with the development of increased resistance in the involved tissue to later invasion by the originally inciting agent? Histologic evidence for the presence of such resistance is given below.

Procedures and Results. Ubiquity of proliferative hyperplasia. Proliferative hyperplastic effects have been reported from other laboratories for a very large number of harmful substances (see ref. 6–20 in present ref. 4). Many of these were also carcinogenic. The ubiquity of this response was further tested here with various selected substances applied to the skin of mice and guinea pigs, as can be seen in Table I. It may be seen that a large number of agents produce hyperplasia and proliferation.

Some characteristics of the hyperplastic response. It seems possible that the proliferative hyperplastic response, as measured by epidermal thickness, is some function of the degree of damage brought about by the agent and therefore some function of the amount of agent used on a given area of skin. As a first step in testing this, it was quickly ascertained with each of the agents tested, including dinitrofluorobenzene (DNFB), that adequately low concentrations, as expected, gave no response whatsoever grossly or histologically in mouse skin.

The suspected relationship was further tested by increasing the amounts of dinitrofluorobenzene (DNFB) applied to mouse skin. This produced only slight epidermal proliferation (max of twofold at 48 hr) with 40 $\mu g/cm^2$ applied once, but it produced four to sixfold epidermal proliferation with 80 $\mu g/cm^2$ applied four times over a 6-hr period (Figs. 1 and 2). This was repeated on ten mice.

If harmful stimuli cause cells to overreplicate and so defy control of growth, then it may be expected that hyperplastic responses will also occur in already proliferated, thickened tissue. Thus repetition of stimulus should lead eventually to extensive proliferative hyperplasia (Figs. 3–5); the latter figure shows guinea pig epidermis thickened 30 fold from DNFB. Figure 6 shows great proliferation of mouse epidermis, produced by daily applications of DNFB over a two-week period. The effect was associated with increase in dermal thickness, very large epidermal cells, and prominent intercellular bridges (Fig. 7). High-enough concentrations of DNFB will produce necrosis (Fig. 8).

However, tissue that is already hyperplastic is much more difficult to damage, necrosis appearing rarely and only with much higher concentrations than the 80 $\mu g/cm^2$ used here. Figures 9 and 10 show this partial damage in the form of cells that are vacuolated and stain weakly, a picture seen in all of the 40 mice tested.

If mice were given only low concentrations of DNFB for some time after the last application of DNFB at high concentration, one may guess that hyperplasia should remain as a reflection of the increased regeneration rate that occurs with continued damage, but it should exist to a much lesser degree than under the more harmful stimulation that obtained before.

To test this, five mice received 14 daily applications of DNFB at 32 $\mu g/cm^2$, and then for two successive days 40 $\mu g/cm^2$. After a one-day rest they were given 16 $\mu g/cm^2$ daily for 19 applications (over 25 days); of the

TABLE I. Proliferative Effect of Selected Substances.

Animal[a]	Substance	Tested property	Range of concentration	Evidence of gross damage	Interval after 1st application, prior to biopsy	Increase in thickness of epidermis
G	Nitric acid	Skin damage	2.0–0.1 N; .25 ml/cm²	++++	50 hr	6 fold
G	Mild soap	Emulsification	16–33 g in 100 g H_2O; 0.5 ml/cm²	0	50 hr	8 fold
G	Harsh soap	Emulsification	16–33 g in 100 g H_2O; 0.5 ml/cm²	±	50 hr	6 fold
G	Salicylic acid[b] (40% in lanolin)	Keratolysis	14 mg in 35 mg lanolin/cm²	+	80–100 hr	3 fold
G	Stripping by cellophane tape	Physical depletion	4–8 times	Loss of tissue	48 hr	3–4 fold
G	Electric clipper	Hair cutting	Once	0	48–72 hr	0
G	Hydrogen fluoride	HF ≅ that released by 750 μg DNFB[c]/cm²	81 μg in 8 mg lanolin/cm²	0	24 hr	2 fold
M	Hydrogen fluoride	HF ≅ 450 μg/cm²	4 μg in 8 mg lanolin/cm²	0	24 hr	0
M	Lanolin daily	Fat of another species	Freely applied	0	27–54 days	+30%
G	DNFB[c]	Immediate reactivity with protein	80 μg in 8 mg lanolin/cm²	++++	5 days	6–8 fold
M	DNFB[c]	Immediate reactivity with protein	80 μg in 8 mg lanolin/cm²	++++	7 days	10–30 fold
G	Tobacco smoke concentrate[d]	Skin damage	100 mg/cm²	++++	3–7 days	10–20 fold

[a] G, guinea pig; M, mouse. Two to ten animals used in each test.
[b] Applied three times over a four-hr period.
[c] Dinitrofluorobenzene.
[d] See text for description of application.

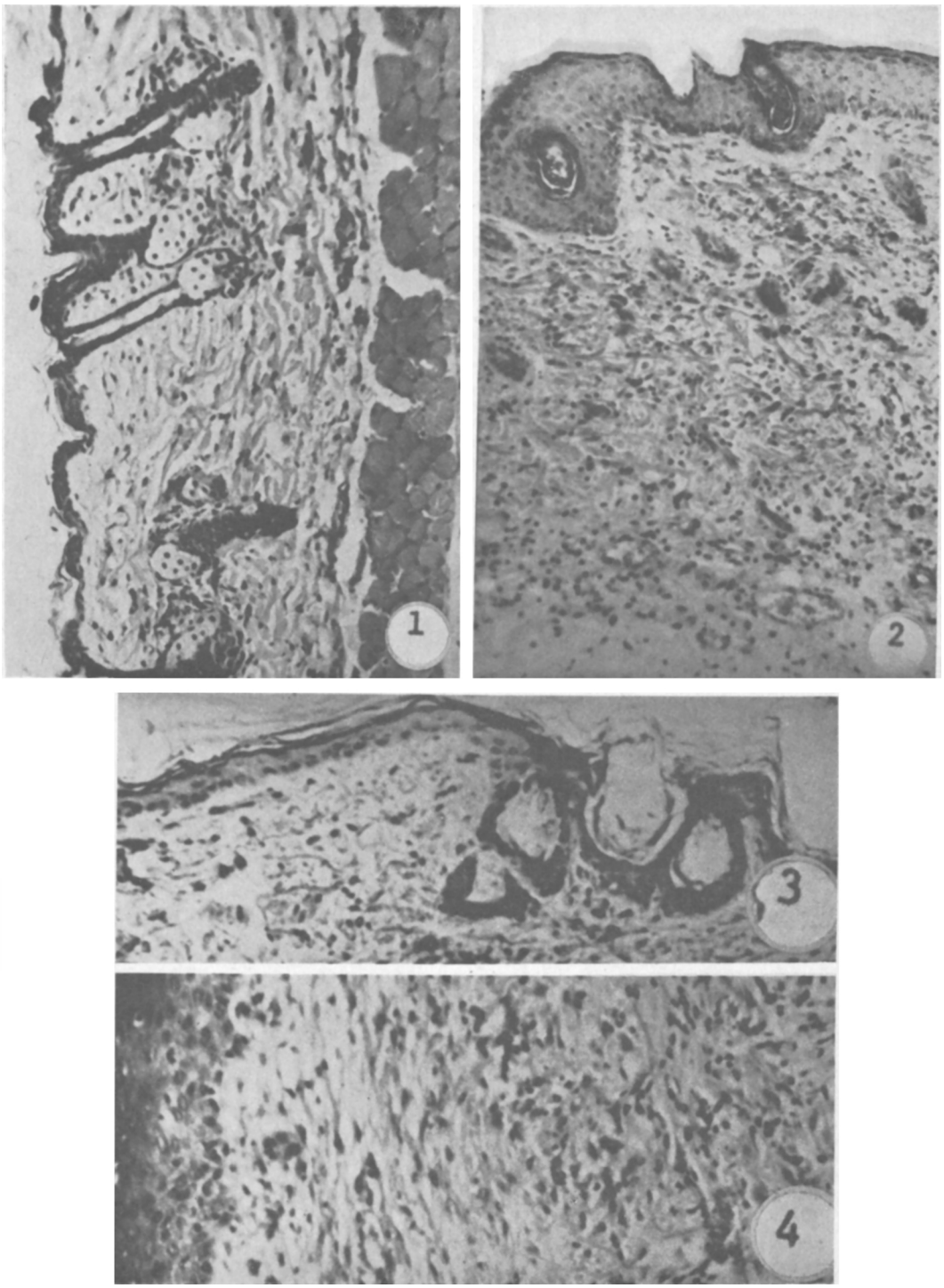


FIG. 1. Normal mouse, ×128.
FIG. 2. Mouse, hyperplasia from DNFB, ×128.
FIG. 3. Guinea pig, normal epidermis, ×128.
FIG. 4. Guinea pig, response to DNFB, ×128.

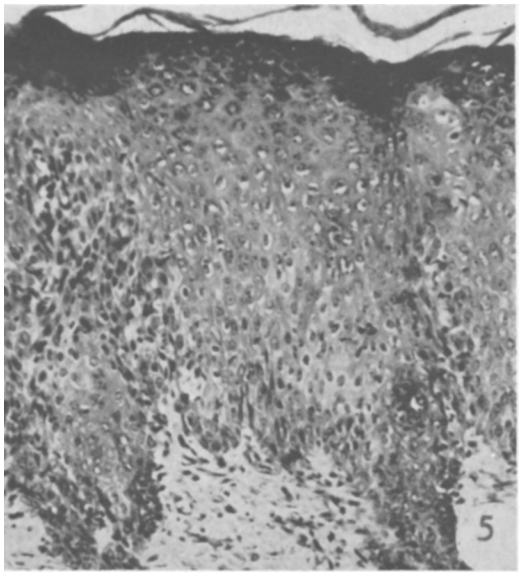


FIG. 5. Guinea pig, response to DNFB, ×128.

five, one was sacrificed after only 15 of the latter applications (21 days). All showed, as expected, only limited hyperplasia of epidermis (two to fourfold thickening) and moderate thickening of dermis, with almost no round cell infiltration or excessive growth of hair follicles. No damage was seen grossly during the period of 25 days, nor microscopically at the end.

Resistance. It seems possible that hyperplastic effects could be associated with an increase in resistance to the inciting agent. The first possible indication of such a response can be seen in Figs. 9 and 10. Resistance might be sought also in the effect of gradually increasing amounts of DNFB, after which it might be possible to administer harmful amounts without producing damage. Twenty mice were treated with daily applications of DNFB of gradually increasing concentration (from 8 to 32 $\mu g/cm^2$), and it was found at the end of the fourth week (after more than a week at 32 μg) that no mice showed evidence of epidermal damage; while 10 mice, used as controls and given the highest concentration (32 μg) from the first day on, showed epidermal ulceration and crusting in every case; in 25% of the cases this occurred within the first week.

Further evidence of the development of resistance was sought with tobacco smoke condensate (SC), obtained from the Tobacco Industry Research Committee. The condensate was applied to the dorsal skin of guinea pigs in order to answer three questions: Can SC be shown to be actually harmful to cells; does it bring about a hyperplastic response; does it produce resistance in previously treated tissues?

From a study of damage to epidermis, the use of SC was given up in mice because of the great systemic toxicity of SC for this animal. On the other hand, for guinea pigs a way had to be found to elicit local damage. By trial and error it was found that gauze impregnated with large amounts of SC and left in contact with the skin would produce severe damage. In one pig, for example, a single application of 60 mg of SC in a gauze circle of radius 0.5 cm produced in 48 hr severe damage and necrosis. All of the epidermis was destroyed and replaced. If the 60-mg amount was again added 4 hr later to the

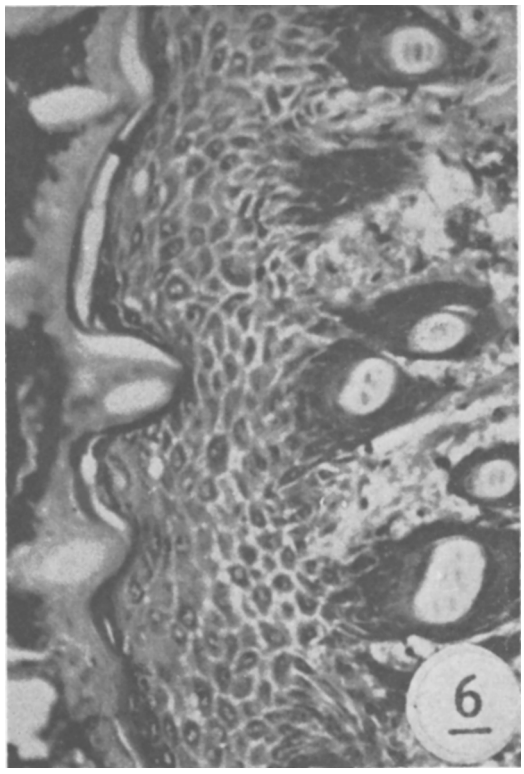
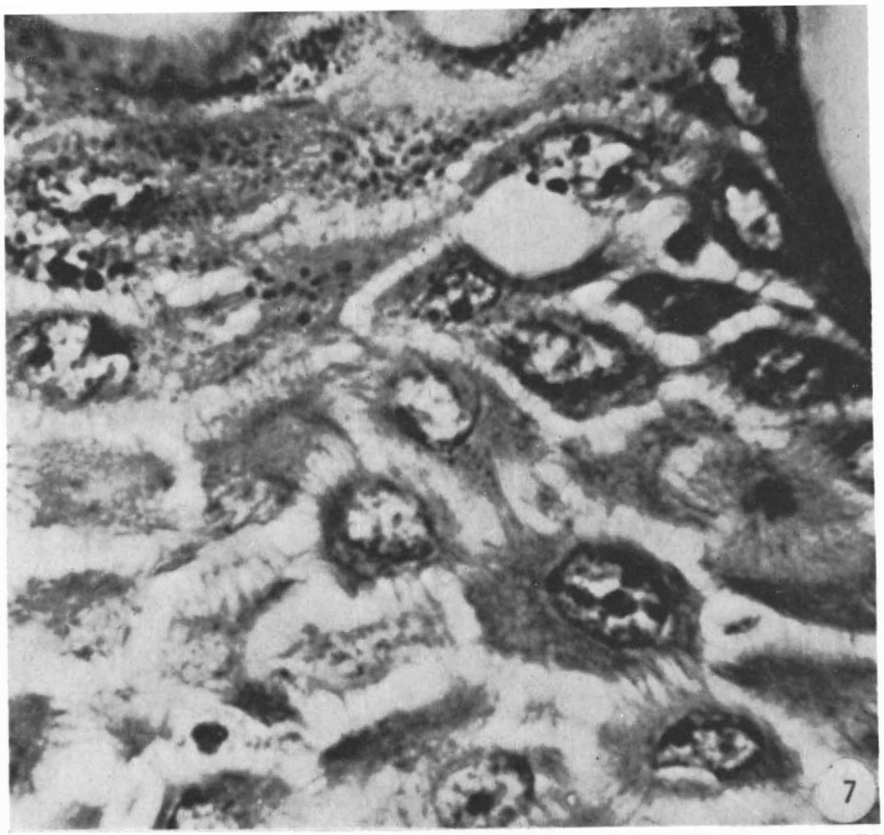


FIG. 6. Mouse, extensive hyperplasia, DNFB, ×128.



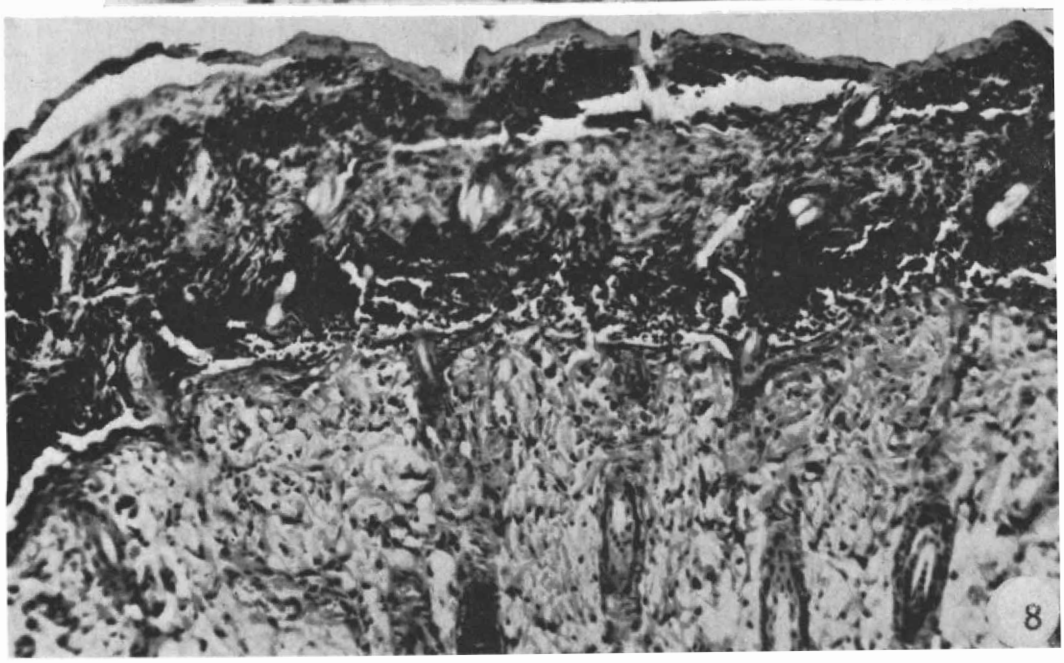


FIG. 7. Mouse, extensive hyperplasia, DNFB, ×1440.
FIG. 8. Mouse, 24-hr effect of very high concentration of DNFB, ×128.

same piece of gauze as the first, deep dermal damage followed with round cell infiltration and no replacement of the necrotic epidermis. There is no question about the ability of SC, in proper concentration and amounts, to damage guinea pig epidermis, since this effect was observed in about 20 animals and never failed to occur.

Nor can there be any doubt about the hyperplastic response that has been reporte previously (5–7) and which always occurred here, except in the last instance cited above in which after a second application, only extreme damage was found. The lowest concentrations (1 mg SC/cm^2), put directly on skin daily for 18 days, produced no gross or histologic evidence of damage, but did result in a threefold thickening of the epidermis, with the usual increase in size of cells and nuclei as well as in number of cells. In the animals given higher amounts, hyperplasia of epidermis was seen regularly, being usually quite extreme. If applied in the gauze, 50 mg of SC produced necrosis and at times fourfold hyperplasia in 48 hr; this is equivalent to the condensate from one quarter of one cigarette.

As for resistance, efforts were made to compare the resistance of two different areas of the skin of one guinea pig pretreated over one of the areas. Tobacco smoke concentrate was applied at various concentrations three times a week for one month to the entire left back. After a 4-day rest the treated and untreated areas of the back were exposed to

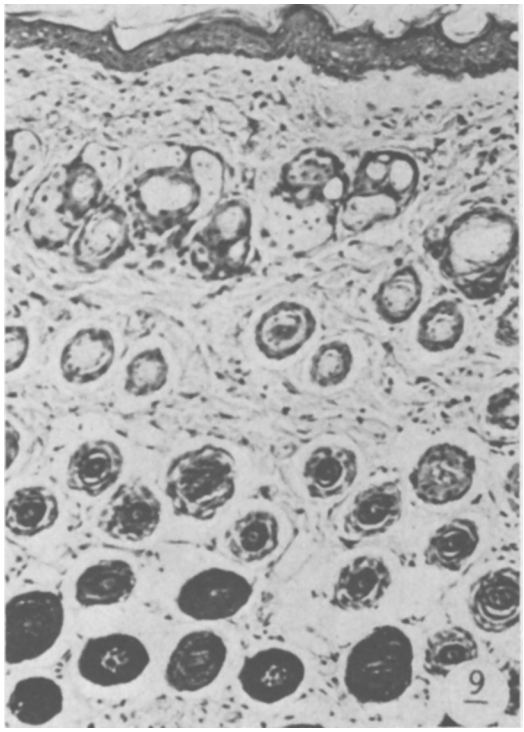


FIG. 9. Mouse, damage in already hyperplastic skin, ×640.

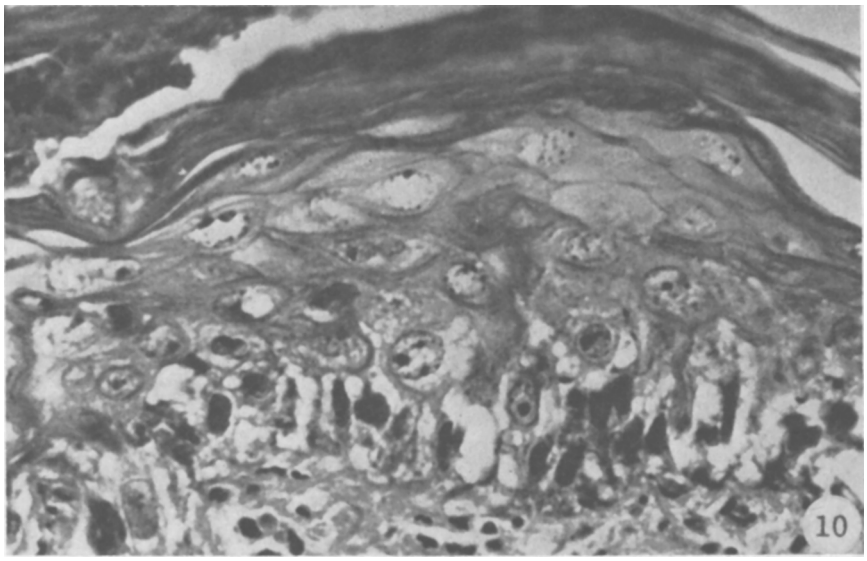


FIG. 10. Same effect as shown in Fig. 9, ×640.

spot tests of 70 mg and 210 mg of SC administered in gauze in individual applications of 10–30 mg over 41 hr. At the end of 41 hr, the lower test concentration showed on the pretreated side hyperplasia of all layers of epidermis and no gross or microscopic damage. The untreated side at this test concentration exhibited necrosis of all epidermis with somewhat hyperplastic underlying dermis and with moderate infiltration of round cells. The epidermis was seen to be separating from the dermis in many places. Significance was gained from use of the same pig. Similar results were obtained with five other pigs.

The higher test concentration on the pretreated side resulted in destroyed epidermis with cell debris on the surface; all was in a somewhat late stage of resolution with extensive epidermal hyperplasia just beneath. The untreated side, however, provided not only a picture of separated, destroyed epidermis but also of intense round cell infiltration; there was dermal but no epidermal hyperplasia.

Further evidence for the resistance being a local phenomenon in the skin was sought as follows. In a normal guinea pig 500 mg of SC was distributed over 48 cm^2 of the left back. This was repeated 1–2 times a day for seven days with a three-day rest period after the fourth dose. Then after five days' rest, the usual tests were applied by SC-impregnated gauze (0.5 cm in radius). After 24 hr of contact with the test gauze, the skin on the untreated side showed evidence of severe damage, considerably less marked at 100 mg than at 180 mg of SC. There seemed to be a rather intermediate degree of damage at 140 mg. There was little round cell infiltration and no successful regenerative response. No debris of necrotic material was seen over the surface.

However, on the prepared, previously treated side a number of differences were observed. At the lower test amount of 100 mg of SC, damaged epidermis was present only as a narrow, partly resolved band of cellular material with much chromatic debris overlying healthy-appearing, hyperplastic epidermis containing many hyperchromatic figures in the basal layer. These were seen especially below the enclosed spaces, which previously were seen so often in hyperplastic tissue (v.s.). At higher amounts, 180 mg of SC, severe damage was found, but unlike the untreated side at this level there was seen new reparative epidermis invading below the necrotic material.

Twenty-four hours after removal of the spot-test material in the same animal the unprepared side still showed extensive damage, while on the previously treated side only healthy, heavily stained thick epidermis was seen.

Quantitative studies, repeated stimuli. After these earlier, orienting studies, more quantitative measurements of the hyperplastic response were made after repetition of a number of damaging stimuli by the same substance. It was expected that the degree of damage from a repeated stimulus would be decreased by the development of resistance and that this might result in a decrease in the hyperplastic response after each later stimulus.

Exposure of five guinea pigs to a succession of equal applications of DNFB at constant intervals of time was followed by measurement of the epidermal thickness of the hyperplastic skin in the area exposed. This was done by making camera lucida drawings of sections and measuring the transposed area with a planimeter. The number of cells was a direct function of thickness (about $\pm$ 10%), as determined by counts of the crowded cells at various stages of response. From Fig. 11, at relatively low concentrations for a guinea pig (90 $\mu g/cm^2$ given at weekly intervals), it may be seen that the immediate effect of each application (except for the first one) was a decrease in epidermal thickness reaching a minimum in about 24 hr, followed by a rapid increase over the next 96 hr to a thickness level far above that present before DNFB was applied. The result of a succession of exposures was an over-all increase in epidermal thickness with time. It may be seen that the hyperplastic effect occurred whether the skin was already hyperplastic or not. Usually about five days after a single stimulus was applied, the thickness of the epidermis subsided at a rate almost equal to that of the increase in thickness

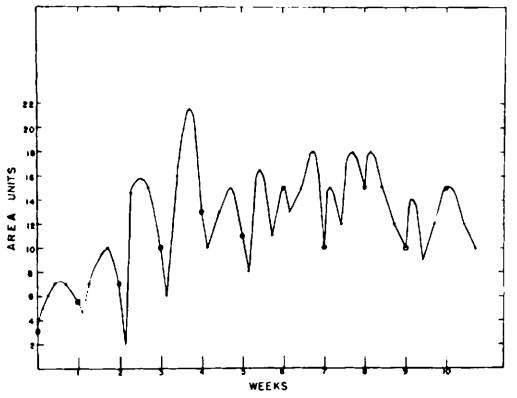


FIG. 11. Quantitative plot of epidermal thickness with time as variable, guinea pig, weekly application DNFB; dot indicates time of application.

during the hyperplastic phase, a rate that by extrapolation would bring the skin back to its preapplication level in about four to five days after the peak was reached. Also, the rate of decrease in thickness immediately after each application of DNFB in a succession of applications is comparable, in this case of 90-μg amounts, to the subsidence rate after each peak response was reached.

After four such exposures to DNFB (Fig. 11) the effect of each application was not so great as before in terms of initial decrease or succeeding increase in epidermal thickness. This suppressed response then remained about the same for the next four exposures, perhaps showing still further suppression and irregularity after the ninth and tenth application.

In Fig. 12, the results obtained from treating the skin of two other pigs, it may be seen that the hyperplastic response is indeed some function of the amount of depletive agent used. It is of interest that the single large amount, (Curve A, Fig. 12), not only gives a much greater hyperplastic response but the response declines at a much slower rate than the other. While some of the increased thickness is made up of widened intercellular spaces, all sections were packed tightly with cells, many of them larger than normal epidermal cells.

Discussion. The evidence reported here, together with that found in the literature, shows that skin of several species responds to application of a large number of harmful substances by hyperplasia and proliferation. The degree of proliferation is some function of the amount of agent applied, both concentration of agent and total amount over a period of time being variables. The effect may be a strictly cellular one since it is not affected by the presence of excessive, proliferated tissue. The response appears to take precedence over the normal control of tissue and cellular growth.

The hyperplastic response of skin tissue is so constant and so easily provoked by a very large number of harmful substances that some common pathway of stimulation of cells to increase their rate of synthesis of intrinsic constituents seems to have come into play. In view of the reparative properties of living systems and the constant uptake of amino acids by cell proteins, it seems possible that this widely occurring hyperplastic response, involving rapid synthesis of complex molecules, is initiated whenever there occurs partial depletion of one or more intrinsic, macromolecular systems in the cell. Once set in motion, the response apparently becomes cell-wide, as shown by the hyperplasia.

So generalized is the effect and so constant the result that it is possible to suggest that a biologic principle, such as the following, is revealed by these responses.

If a harmful agent interacts with one or more intrinsic constituents (i.e., targets) of a cell, so producing partial depletion (perhaps only functional) of those constituents, the cell will respond by synthesis of more of

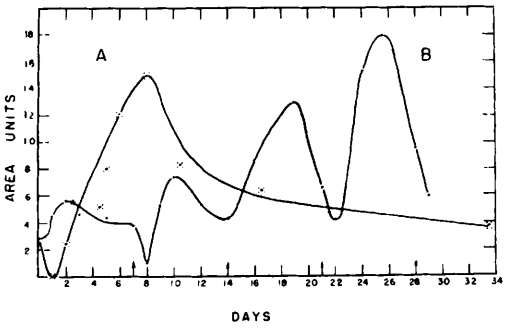


FIG. 12. Same kind of plot, effect of weekly applications compared with that of a single highly concentrated dose.

those depleted substances, not only replacing the amounts lost but also producing as a function of the degree of depletion an excess of targets as well as of other intrinsic substances. Intrinsic cellular substances may be defined for these purposes as proteins, polysaccharides, and other macromolecular substances synthesized within the cell. They may be defined for these purposes as proteins, polysaccharides, and other macromolecular substances synthesized within the cell. They do not include fuel substances, amino acids, vitamins, or ions, brought to the cell from the environment.

In defying the control of growth and form of the biologic system, the mechanism appears to be some form of adaptation of high priority. In fact, since cells from earliest times must have evolved mechanisms to respond strongly to depletive stimuli; this one of increased and excessive synthesis may be quite primitive. A simple theory of biologic response to partial depletion has been announced (4), which provides a basis, although not a mechanism, for the hyperplastic response. Evidence in this paper gives some histologic support for the theory. There may be possible implication in this for the development of the cancer cell.

Besides repletion of lost constituents, a further possible biologic advantage of a cell's regenerative response might be that of increased resistance, as suggested by the finding here reported of greatly increased local resistance to the agent used. This may be accomplished by various means but a possible one could be simply the provision of an excess of cellular target to combine with the depletive agent if and when it were to invade the cell again. A basis for this idea is derived in the above theory (4), leading also to a new explanation of antibody formation.

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Measles Antigen and Syncytium Formation in Brain Cell Cultures from Subacute Sclerosing Panencephalitis (SSPE)* (33377)

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Subacute sclerosing panencephalitis (SSPE) is a progressive degenerative, neurologic disease of children that begins with mental and motor deterioration. After an interval of weeks or months, a state of plastic rigidity, interrupted by myoclonic jerks, intervenes. Coma, with signs of decortication and often of hypothalamic dysfunction, occurs during the terminal stage of this fatal illness.

Observation of cytoplasmic and nuclear inclusion bodies in cortical neurons of patients with SSPE led Dawson to propose a viral etiology for this disease (1). Evidence for an association between SSPE and measles virus has been reviewed and includes: a history of measles infection prior to the onset of symptoms; demonstration by electron microscopy of structures in the diseased brain that are morphologically similar to the nucleocapsids

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