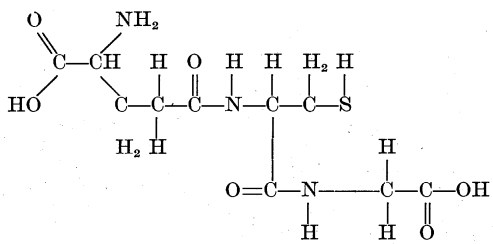
Comments and Communications

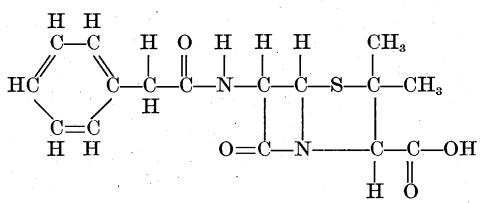
In Vitro Production of Cortisone

IN YOUR issue of November 3, 1950 (*Science,* **112**, 524), there is a most interesting account by Dr. Seneca *et al.* of the production *in vitro* of cortisone from desoxycorticosterone by various mammalian tissues, and especially by the adrenal gland. I believe, however, that in explaining the observed facts the authors have overlooked one most important thing, namely, that to every flask they had added 10,000 or 50,000 units penicillin G and 3.34 ml propylene glycol. In all instances where, moreover, glutathione had been added, the results were consistently negative.

The fact that in the flasks with penicillin but without glutathione nearly always the formation of cortisone by adrenal tissue was demonstrable, whereas this was consistently lacking in the flasks containing the same ingredients plus glutathione, seems to indicate that in the latter case glutathione inhibited cortisone formation. Now glutathione



and penicillin G



have so large a part of their structures in common (*cf.* E. Fischer, *Science,* **106**, 146 [1947]) that competitive inhibition becomes a possibility. And, if so, the preliminary conclusion should be that it is penicillin G that is the active catalyzer of the formation of cortisone in these experiments—a point well worth further experimental confirmation in order to promote both a deeper insight into the mode of action and the chemical potentialities of penicillin, on one hand, and perhaps the artificial preparation of glucocorticoids from readily available desoxycorticosterone, on the other.[1] But at the same time we must realize that these experimental results are not to be considered as experimental evidence in support of Lewin and Wassen's views, for in the living body no penicillin is present unless expressly administered. On the other hand, penicillin may then be a powerful adjuvant to their method of treatment of rheumatoid arthritis with desoxycorticosterone acetate plus ascorbic acid, as well as of the same mode of treatment in psychiatry proposed by M. Möller (*Svenska Läkartidn.,* **43**, 475 [1946]); R. Jens (*Northwest Med.,* **48**, 609 [1949]); E. H. Cranswick and T. C. Hall (*Lancet,* **I**, 540 [1950]); G. Fachini, F. Ceresa, E. Morpurgo, and Z. Korenyi (*ibid.,* 734); and H. Bourne (*ibid.,* 925). Perhaps the therapeutic success of these methods may thus be made more convincing.

[1] A further implication may be that the rapid clinical recovery from infections by penicillin may be in part due to the bacteriostatic action of the antibiotic, but in part also to a rapid readaptation in the sense of H. Selye, by the increased production of glucocorticoids. This "aspecific" effect of penicillin also merits further investigation.

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WE HAVE read with interest Dr. Grevenstuk's comments on our paper dealing with the *in vitro* production of cortisone by mammalian cells (*1*). It is our opinion, however, that lack of experimental evidence does not warrant Dr. Grevenstuk's conclusions concerning the role played by penicillin.

Penicillin was apparently not included in the systems employed by McGinty *et al.* (*2*), when they incubated Compound S with adrenal homogenates, and isolated Compound F, a substance very closely related to cortisone. Neither did the workers at the Worcester Foundation, apparently, employ penicillin when they produced corticosterone by the method of adrenal perfusion (*3*).

Dr. Grevenstuk's suggestion that penicillin therapy in infectious diseases involves a readaptation in the sense of H. Selye might be valid. It may readily explain the favorable results obtained in some cases of rheumatoid arthritis when treated with gold salts; this might be thought of as an activation of the adrenal cortex to produce glucocorticoids. No evidence, however, exists as yet showing that penicillin has any stimulating effect on the adrenal cortex in normal animals.

Although the formulas of penicillin and glutathione (GSH) may be written to resemble each other, there appears little evidence that any definite biological relation exists between these two compounds. The reference cited by Dr. Grevenstuk (Fischer) states: ". . . It would be too far-reaching to draw, without experimental basis, any conclusion from this circumstance [resemblance of the formulas of penicillin and GSH], but one may think of the possibility of penicillin competing with glutathione for enzymatic or other mechanisms important for *microbial* [italics ours] reproduction." There are some clinical observations on a few cases in which penicillin injections appeared to have caused a transient rise in the blood level of GSH but did not change the blood level of oxidized glutathione (GSSG) (*4, 5*). This fact in

itself might arouse suspicion, especially when another investigator claims that GSSG acts as an "H-acceptor" after penicillin injections (*6*). In a study carried out with microorganisms, it was concluded that "such simple experiments do not themselves afford unequivocal proof of the participation of glutathione in the mechanism of penicillin action. However, it is *generally assumed* [italics ours] that – SH groups are involved" (*7*). Penicillin was found to inhibit the enzymic hydrolysis of GSH, but this inhibition could be overcome by the addition to the medium of more GSH and glutamine (*8*). If the – SH groups of GSH would be blocked, then the addition of GSH to our medium could not be expected to inhibit the oxidation-reduction system postulated by us. It certainly could not inhibit the synergism caused by insulin. The complete inhibition whenever an excess of GSH was added may be better explained by the well-known inactivation of insulin in the presence of GSH, or by its ability to keep the added vitamin C or other still-unidentified factors in their reduced states.

Penicillin is known to be inactivated rapidly at 37° C, the temperature at which all our experiments were carried out, but the nature of the inactive end product is not known, nor is it definitely established by what mechanism the bactericidal action of penicillin proceeds. The hypothesis is advanced that some assimilatory processes are blocked at the cell walls, such as the assimilation of glutamic acid (*9*). One might speculate that some of the biological activity of penicillin is related to its optical configuration, which happens to be the "unnatural" one, and that penicillin may be able to enter metabolic process in microorganisms capable of metabolizing compounds having the D-configuration. Whether such reasoning still holds for mammalian cells is debatable. Many enzyme preparations from mammalian cells, for instance, serve usefully in the enzymic resolution of amino acid racemates.

It is very dangerous to compare *in vitro* experiments with clinical cases, because of the extreme differences in conditions. Clinically, the favorable results obtained in cases of rheumatoid arthritis with DOC plus vitamin C are open to question, and, according to the most recent clinical findings, previous favorable results have not been confirmed. It is therefore fallacious to advocate clinical trials involving penicillin as an adjuvant to DOC plus vitamin C on the basis of speculative inferences drawn from *in vitro* experiments. The favorable results obtained by the combined use of DOC plus vitamin C in psychiatric cases are also being questioned. Clinically, the combined cortisone and insulin shock therapy in psychiatric treatment gave immediate favorable results in about 50% of the cases (*10*), but the final result was not different from insulin shock therapy alone after discontinuance of cortisone.

In a preliminary report on clinical trials (*11*) it was shown that the administration of insulin reduced the cortisone requirement in the treatment of rheumatoid arthritis in all stages by as much as 75% of the dosages usually given, and that this type of therapy apparently eliminated all the usual objectionable side effects of cortisone. None of these patients had received penicillin at any time immediately preceding this type of therapy.

These are but a few of the reasons why we have not taken penicillin into consideration as an active participant in our system. We believe that penicillin serves no other purpose than to keep our system free of bacterial contaminants, and that any other conclusions drawn from our experiments with respect to the action of penicillin cannot be supported on the basis of experimental facts available up to date.

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References

1. SENECA *et al. Science*, **112**, 524 (1950).
2. MCGINTY *et al. Ibid.*, 506.
3. HECHTER *et al. J. Am. Chem. Soc.*, **71**, 3261 (1949).
4. MOSONYI and OBLATT. *Orvosi Hetilap*, **89**, 429 (1948).
5. BUTTITTA and DUCHALIOT. *Boll. soc. ital. biol. sper.*, **23**, 655 (1947).
6. MULÉ. *Experientia*, **3**, 292 (1947).
7. PRATT and DUFRENOY. *J. Am. Chem. Soc.*, **70**, 1671 (1948).
8. BINKLEY and OLSON. *J. Biol. Chem.*, **188**, 451 (1951).
9. GALE and TAYLOR. *J. Gen. Microbiol.*, **1**, 314 (1947).
10. SENECA. Unpublished observations.
11. HENDERSON *et al. J. Clin. Endocrinol.*, **10**, 800 (1950).

The Monarch Butterfly

IN YOUR pages (*Science*, **113**, 68 [1951]; see also p. 729) there was published an article by W. D. Field, J. F. Gates Clarke, and J. G. Franclemont on the Commission's decision that in future the name *Papilio plexippus* Linnaeus (1758) shall apply to the butterfly known in America as the Monarch, thus putting an end to a controversy that has troubled lepidopterists for at least a generation.

In the Minutes of the Paris Meeting of the Commission, this decision is recorded in the following words:

(1) to use their plenary powers to direct that the trivial name *plexippus* Linnaeus, 1758 (as published in the binomial combination *Papilio plexippus*) should be applied to the American species figured as *Danais plexippus* by Holland (W. J.), 1931, *Butterfly Book* as figure 1 on plate 7;

(2) to place the name . . . as determined in (1) above on the *Official List*. . . .

(3) to render an Opinion setting out the decisions recorded in (1) and (2) above.

This statement, for which I take my full share of responsibility, means no more, and no less, than it says. It does not fix any "type" specimen or "type" figure of *plexippus* and it does not imply that the figure to which reference is made belongs to any particular subspecies of *plexippus* from any particular "type" or other locality. It says, in effect, only that