severe rejection crises, J.R. had mild but definite signs of rejection, probably starting on day 36. Several days before the apearance of clinical evidence of rejection, there was a significant increase in the number of large cells plus mitoses in PHA-stimulated cultures of her lymphocytes. Raising the dosage of immunosuppressive drugs decreased the response in vitro and eliminated signs of rejection.

This technique of lymphocyte culture offers a new approach to the problem of donor and recipient typing for renal homotransplantation. It may also provide a way of assessing the degree of immunologic suppression effected by cytotoxic agents. Use of this technique should allow further insight into the problem of histocompatibility and the nature of drug-induced suppression of the immune response.

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References and Notes

1. J. Hastings, S. Freedman, O. Rendon, H. L. Cooper, K. Hirschhorn, *Nature* **192**, 1214 (1961).
2. K. Hirschhorn, F. Bach, R. L. Kolodny, I. L. Firscheim, N. Hashem, *Science* **142**, 1185 (1963).
3. F. Bach and K. Hirschhorn, this issue.
4. K. Hirschhorn and F. Bach, unpublished data; B. Bain, M. R. Vas, P. H. Lowy, *Blood* **23**, 108 (1964).
5. L. Brent and P. O. Medawar, *Brit. Med. J.* **1963-II**, 269.
6. K. H. Stenzel and A. L. Rubin, unpublished data.
7. This work was supported in part by grants from the U.S. Public Health Service (HE 08736 and HD00542), the American Heart Association, the Health Research Council of the City of New York, and the Association for the Aid of Crippled Children.

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Glycogen Synthetase: Its Response to Cortisol

Abstract. *Glycogen synthetase activity in the livers of starved mice is stimulated by the glucocorticoid, cortisol.*

Greengard *et al.* (*1*) and Weber *et al.* (*2*) recently reported that liver glycogenesis induced by cortisone in starved rats is inhibited by puromycin and actinomycin, two agents that interfere with protein synthesis. It was concluded that the formation of an enzyme protein induced by cortisone and required for glycogenesis is interrupted. The enzymes investigated were aldolase, lactic dehydrogenase, glucose-6-phosphatase, fructose-1,6-diphosphatase, and tyrosine transaminase. In addition, transaminase has been implicated in cortisol-induced glucose precursor synthesis by Rosen *et al.* (*3*).

As part of a study (*4*) being conducted on hepatotoxic agents and glycogenesis, we found that yet another enzyme, glycogen synthetase (uridine-diphosphate-glucose–glycogen-1,4-transglucosylase) is stimulated in vivo by the glucocorticoid, cortisol. This observation now becomes relevant to the question of the identity of the enzyme protein whose antibiotic-inhibited synthesis prevented glycogenesis by cortisone.

Three-month-old A/Jax male mice (20 to 25 g) in groups of six were killed at the intervals indicated in Table 1. A 10-percent homogenate of the freshly excised liver in each case was prepared in a medium composed of 0.25*M* sucrose, 0.001*M* EDTA, and 0.1*M* NaF solution. The homogenate was assayed for glycogen synthetase according to Leloir and Goldemberg (*5*). Liver glycogen was extracted from a weighed specimen of fresh liver with hot 30 percent KOH solution. It was precipitated by ethanol and measured as glucose units by Dische's cysteine-sulfuric acid reaction (*6*).

Table 1. Liver glycogen and glycogen synthetase during starvation; effects of cortisol and deoxycorticosterone acetate (DOCA).

Expt. No.	Fasting interval (hr)	Steroid (1 mg)	Glycogen (g/100 g liver) (Mean±S.D.)	Glycogen synthetase* (specific activity) (Mean±S.D.)	Comparison of Expt. No.	Comparison of Expt. No.	*P*
1	0		3.35 ± 0.35	2.02 ± 0.13			
2	12		0.05 ± 0.04	1.18 ± 0.04	2	1	<0.01
3	24		0.05 ± 0.01	1.20 ± 0.20	3	1	<0.01
4	12	Cortisol	3.02 ± 0.32	1.70 ± 0.11	4	2	<0.01
5	12	DOCA	0.02 ± 0.02	1.09 ± 0.03	5	2	>0.05
6	24	Cortisol	0.69 ± 0.39	1.77 ± 0.11	6	3	<0.01
7	24	Cortisol (last 12 hours)	2.70 ± 0.63	1.86 ± 0.21	7	3	<0.01

* Micromoles of uridine diphosphate formed per hour per milligram of protein in the presence of glucose-6-phosphate as described by Leloir and Goldemberg.

Table 1 shows that (i) liver glycogen depletion by 12 and 24 hours of fasting is accompanied by a significant reduction in glycogen synthetase (expts. 1, 2, and 3); (ii) the administration of cortisol (expt. 4) but not deoxycorticosterone acetate (expt. 5) prevents this phenomenon at 12 hours; (iii) 24 hours after administering cortisol, the glycogen value is depressed but glycogen synthetase activity is not; and (iv) the administration of cortisol at the 12-hour interval restores the values of both glycogen and glycogen synthetase to normal in 24 hours.

The present results agree with the conclusion of Dorsey and Munck (*7*) that changes in blood glucose level alone in fasted adrenalectomized rats cannot explain quantitatively the rate of deposition of glycogen soon after cortisol injection. The alternatives suggested were that cortisol either (i) provided more substrate in the form of blood glucose, (ii) acted directly on the liver by mechanisms such as enzyme activation, changes in physiology of circulation, and so forth, or (iii) acted directly on extra-hepatic tissues to produce glucose, activator, and substrate. Our findings support a direct action of cortisol on the liver.

It is clear that the activity of glycogen synthetase is influenced by glucocorticoid and not by mineralocorticoid. Moreover, a decrease in glycogen concentration is not always accompanied by a drop in glycogen synthetase (expt. 6). The central role of glycogen synthetase in glycogenesis, therefore, justifies its inclusion in evaluations of individual enzymes responding to cortisol stimulation much along the lines reported by Hilz *et al.* (*8*).

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References and Notes

1. O. Greengard, G. Weber, R. L. Singhal, *Science* **141**, 160 (1963).
2. G. Weber, R. L. Singhal, N. B. Stamm, *ibid.* **142**, 390 (1963).
3. F. Rosen, N. R. Roberts, C. A. Nichol, *J. Biol. Chem.* **234**, 476 (1959).
4. H. G. Sie, *Federation Proc.* **22**, 585 (1963); ———, *Biochim. Biophys. Acta*, in press.
5. L. F. Leloir and S. H. Goldemberg, *J. Biol. Chem.* **235**, 919 (1960).
6. Z. Dische, *ibid.* **181**, 379 (1949).
7. J. L. Dorsey and A. Munck, *Endocrinology* **71**, 605 (1962).
8. H. Hilz, W. Tarnowski, P. Arend, *Biochem. Biophys. Res. Commun.* **9**, 492 (1963).

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Inhibitory Postsynaptic Potentials in Grasshopper Muscle

Abstract. *Hyperpolarizing inhibitory postsynaptic potentials have been discovered in fibers of the "jumping" muscle of the grasshopper. These potentials attenuate the depolarizing excitatory postsynaptic responses. They are enhanced during depolarization of the muscle fiber with applied current and are diminished and then reversed during hyperpolarization. The electrogenesis appears to be caused by chloride-activation. Gamma-aminobutyric acid activates the inhibitory synaptic membrane and picrotoxin is an inactivator agent.*

The occurrence of inhibitory neuromuscular synapses in the Crustacea is well established, but evidence for peripheral inhibitory activity in the other large arthropod group, the Insecta, has been slight (*1*). However, during an examination of neuromuscular transmission in the metathoracic extensor tibiae muscle of the locust after section of its motor innervation ("denervation"), potentials were discovered (*2*) that appeared to be hyperpolarizing inhibitory postsynaptic potentials. Hyperpolarizing potentials had previously been recorded from this muscle by Hoyle (*3*), but they did not seem to attenuate the depolarizing excitatory potentials. In an attempt to resolve these conflicting observations the properties of the hyperpolarizing postsynaptic potentials of normal preparations have been reexamined.

The metathoracic extensor tibiae muscle of the lubber grasshopper, *Romalea microptera*, was used throughout this investigation. Recordings of inhibitory postsynaptic potentials were obtained from about 70 individual specimens. Most recordings were restricted to the closely packed, highly tracheolated, bundle of muscle fibers at the proximal end of the muscle. These fibers, most of which have smaller diameters than the rest of the fibers of the extensor muscle, are either triply innervated, receiving endings from a "fast" and a "slow" excitor axon as well as from the "hyperpolarizer" or inhibitor axon, or they are dually innervated, with no endings from the "fast" axon. Although the "slow" excitor and the inhibitor axons originate from the metathoracic ganglion (*3*) in the same fine nerve (No. 3b) and cannot be separated by dissection, the two axons usually have slightly different thresholds to electrical stimulation and can therefore be excited independently (Fig. 1*A*). Reflex excitation of the two axons obtained by stroking different parts of the animal with the tip of a fine brush has frequently been used as a further method of analyzing the two synaptic events, since by selective activation of different reflex pathways the two axons can be excited independently or simultaneously (Fig. 1, *B–D*).

The largest hyperpolarizing postsynaptic potentials (about 15 mv) are recorded from fibers with relatively low resting potentials (−35 to −45 mv), whereas only very small hyperpolarizing postsynaptic potentials are observed in fibers with resting potentials greater than −65 mv. The hyperpolarizing postsynaptic potentials summate (Fig. 1, *B* and *C*) and facilitate at high frequencies of stimulation, these effects being most obvious in fibers with low resting potentials. The "slow" excitatory potentials, which produce depolarizations between 1 and 30 mv, are modified by the hyperpolarizing electrogenesis. This excitatory potential is attenuated if it arises during the early phase of the hyperpolarization (Fig. 1*A*). Thus, such an attenuated potential may indeed be termed an inhibitory postsynaptic potential. However, if the excitatory potential arises during the declining phase of the inhibitory potential, the excitatory is usually slightly enhanced. Presumably, therefore, the active phase of the inhibitory electrogenesis is relatively brief compared with the duration of the inhibitory potential which it evokes, the declining phase of the inhibitory potential being passive. The discrepancies between the present results and those obtained by Hoyle (*3*) could be explained on the basis that he examined the effects of the passive and not the active phase of the inhibitory postsynaptic potential on the excitatory electrogenesis. This would account not only for the apparent lack of attenuation of the excitatory potentials by the inhibitory potential, but would also explain the slight enhancement of the "fast" excitatory response that he observed.

The inhibitory postsynaptic potential is augmented during depolarization of the muscle fiber by applied current (Fig. 2, *C* and *D*), while reversal of the response occurs during hyperpolarization of the muscle fiber (Fig. 2, *A* and *B*). The reversal or equilibrium potential for the inhibitory potential is usually between −65 and −75 mv, but in some

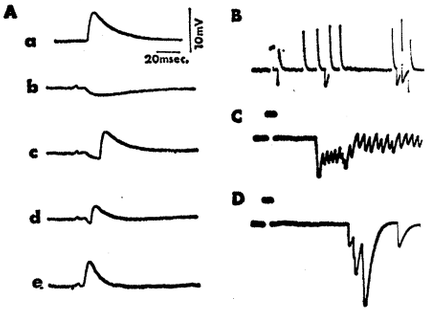

Fig. 1. Hyperpolarizing inhibitory and depolarizing excitatory postsynaptic potentials recorded from fibers of the metathoracic extensor tibiae muscle of *Romalea microptera*. *A*, Recordings from a single fiber. *a* and *b*, Control responses to reflex stimulation of the "slow" axon (*a*) and to electrical stimulation of the inhibitory axon (*b*). *c–e*, Interaction between excitatory and inhibitory postsynaptic potentials as intervals between responses are changed. The excitatory potential was not attenuated when it occurred after the peak of the inhibitory postsynaptic potential (*c*). Only the falling phase of the excitatory potential was affected when the inhibitory potential began later than did the excitatory potential (*e*). *B–D*, Postsynaptic potentials evoked by reflex stimulation recorded from three different fibers which had low resting potentials (−40, −42 and −45 mv, respectively). In *B* both excitatory and inhibitory potentials were evoked, the first response being an inhibitory potential which diminished an excitatory potential that followed it closely. Note marked summation of the inhibitory potentials in *C* and *D*. Calibrating pulses (5 mv and 100 msec) appear at the beginning of each trace.