has reported finding greatly increased in vivo incorporation of thymidine into the DNA of mouse spleen cells after injection of PHA. In view of the inverse relationship observed between the antibody-forming and DNA-synthesizing capacity of immune cells[17] cellular synthetic activities instigated by PHA could prove to be incompatible with productive antibody.

Studies done by GOWANS[18] showed that injected spleen cells rapidly changed into large pyroninophilic cells which then proceeded to divide in the host. Similar morphological results were obtained in vitro by HIRSCHHORN[19] with PHA and with specific antigen. The failure to produce a GVH reaction with PHA-stimulated spleen cells in vivo and in vitro in the present experiments suggests that cells once stimulated, even non-specifically, lose their ability to respond to a second stimulus with host antigens. Thus blast cell transformation may be connected with immunologic inactivation of the lymphoid cells, a 'sterile activation', as it were, through the mediation of PHA.

Since the GVH reaction is achievable only through immunocompetent cells, the findings here would suggest that blastogenesis may result in the production of cells no longer competent to initiate this reaction. Further studies in our laboratory will attempt to explore the possibility that specific blast transformation may also be productive of a cell incompetent to induce the GVH reaction[20].

Zusammenfassung. Mit Phytohämagglutinin behandelte Milzzellen erwachsener Mäuse, übertragen auf neugeborene Balb/c-Mäuse, lösen die erwartete «Runt-disease» nicht aus. Versuche mit Milzzellsuspensionen werden diskutiert.

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University of Oregon Medical School, Portland (Oregon 97201, USA), 2 May 1968.

[17] O. MÄKELÄ and G. J. V. NOSSAL, J. exp. Med. *115*, 231 (1962).
[18] J. L. GOWANS, B. M. GESNER and D. D. MCGREGOR, in *Biological Activity of the Leucocyte* (Eds G. E. W. WOLSTENHOLME and M. O'CONNOR; Churchill, London 1961), p. 32.
[19] K. HIRSCHHORN, F. BACH, R. L. KOLODNY, I. L. FIRSCHEIN and N. HASHEM, Science *142*, 1185 (1963).
[20] This investigation was supported by U.S. Atomic Energy Commission, contracts Nos. RLO-1927-26 and AT (45-1)-581.

The Effect of L-Thyroxine on the Absorption of Calcium and Strontium

It is now becoming increasingly clear that in experimental animals the absorption of calcium and strontium is under metabolic control at least at 2 levels in the gastro-intestinal tract. Firstly by the calcium-specific active transfer process located in the duodenum[1] and secondly, by a metabolically dependent block which limits the passage of calcium and strontium from the lumen of the lower small intestine[2,3]. Both these processes are dependent on oxidative phosphorylation and can be inactivated by certain metabolic inhibitors[3,4].

The thyroid hormones have been shown to uncouple oxidative phosphorylation[5] and a number of manifestations of impaired calcium metabolism have been reported in cases of human hyperthyroidism[6–9]. In vitro studies of calcium transfer across everted duodenal sacs prepared from triiodothyronine- or thyroid stimulating hormone-treated rats showed a marked depression of the duodenal active transport of calcium[10]. This observation led to the suggestion that calcium absorption may be depressed in human hyperthyroidism. In this report it is shown that the overall absorption of both calcium and strontium is in fact increased in rats treated with thyroxine.

The experimental procedures have been described in detail elsewhere[3,11]. Female rats of the highly inbred August strain, aged 6–8 weeks, were given 1 mg L-thyroxine s.c. for 3 days. After fasting overnight the rats were given a single oral dose of 0.5 μCi$^{47}CaCl_2$ plus 0.5 μCi$^{85}SrCl_2$ and killed 7 h later. Absorption was estimated from the amount of each nuclide retained in the whole body less the amount retained in the gastro-intestinal tract.

The results presented in the Table show that in thyroxine-treated rats there is an increased absorption of both calcium and strontium. It is suggested that this increased absorption results from inhibition of the metabolic block in the small intestine, due to uncoupling of oxidative

Effect of L-thyroxine on the absorption of calcium and strontium by the rat

Treatment	Percentage of dose absorbed from G.I. tract in 7 h	
	Calcium-47	Strontium-85
None	[a] 56.6 ± 2.3 (13)	26.7 ± 1.0 (22)
L-Thyroxine 1 mg/d for 3 days	77.6 ± 2.2 (8)	46.2 ± 3.0 (17)

No. of animals in brackets. [a] Mean ± standard error of the mean.

[1] D. SCHACTER, E. B. DOWDLE and H. SCHENKER, Am. J. Physiol. *198*, 263 (1960).
[2] R. H. WASSERMAN, Nature *201*, 997 (1964).
[3] D. M. TAYLOR, in *Strontium Metabolism* (Eds. J. M. A. LENIHAN, J. F. LOUTIT and J. H. MARTIN; Academic Press, London 1967), p. 175.
[4] D. V. KIMBERG, D. SCHACTER and H. SCHENKER, Am. J. Physiol. *200*, 1256 (1961).
[5] A. L. LEHNINGER and B. L. RAY, Science *125*, 748 (1957).
[6] J. C. AUB, W. BAUER, C. HEATH and M. ROPES, J. clin. Invest. *7*, 7 (1929).
[7] P. B. COOK, J. R. NASSIM and J. COLLINS, Q. Jl Med. *28*, 505 (1959).
[8] W. BORTZ, E. EISENBERG, C. Y. BOWERS and M. PONT, Ann. Intern. Med. *54*, 610 (1961).
[9] C. R. KLEEMAN, S. TUTTLE and S. H. BASSETT, J. clin. Endocr. Metab. *18*, 477 (1958).
[10] J. A. FRIEDLAND, G. A. WILLIAMS, E. N. BOWSER, W. J. HENDERSON and E. HOFFEINS, Proc. Soc. exp. Biol. Med. *120*, 20 (1965).
[11] D. M. TAYLOR, P. H. BLIGH and M. H. DUGGAN, Biochem. J. *83*, 25 (1964).

phosphorylation by thyroxine, thus allowing greater amounts of calcium and strontium to cross the intestinal wall.

The relative importance of the duodenal active transfer mechanism and the metabolic block in the small intestine in the control of calcium and strontium absorption is not yet understood. However, the fact that the duodenal mechanism is markedly depressed in hyperthyroidism while overall calcium and strontium absorption is increased suggests that the metabolic block in the small intestine exerts the greatest influence on the absorption of these metals.

The presence of the duodenal active transport mechanism and the metabolic block to calcium and strontium transfer from the lumen of the small intestine have not yet been demonstrated in man. However, if they are present in the human intestine it seems likely that calcium, and strontium, absorption will be increased in hyperthyroidism.

Résumé. Chez le rat, l'administration de L-thyroxine pendant les 3 jours précédant un dosage oral de ^{47}Ca et ^{85}Sr provoque une augmentation frappante de l'absorption des isotopes. On suggère que cette augmentation est le résultat de l'inhibition du bloc métabolique au passage du calcium et du strontium à travers l'intestin grêle par non assemblage de la phosphorylation oxydative par la thyroxine.

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On the Innervation of the Prothoracic Glands in *Papilio demoleus* L. (Lepidoptera)

Innervation of the prothoracic glands has been reported in at least 5 insect orders (Apterygota, Orthoptera, Hemiptera, Coleoptera and Lepidoptera). But in many of these cases, the observations of one investigator have been contradicted by those of another, sometimes within the same species. In *Tenebrio molitor*, for example, ARVY and GABE[1] report innervation of the glands, SRIVASTAVA[2] contradicts them. In *Hyalophora cecropia*, WILLIAMS[3] has reported profuse innervation, while HERMAN and GILBERT[4] find it 'much more difficult to get a conclusive histological evidence of the same'. The main reason for these conflicting observations has been the fact that they are based on gross dissections alone and no attempt has been made to stain the nerves histologically or in situ.

We, therefore, attempted to stain the nerves in situ by employing the intra vitam leucomethylene blue nerve staining technique of ZACHARUK (as described by STAY and GELPERIN[5]) in the fifth instar larva of *Papilio demoleus*. The staining reveals that the prothoracic glands

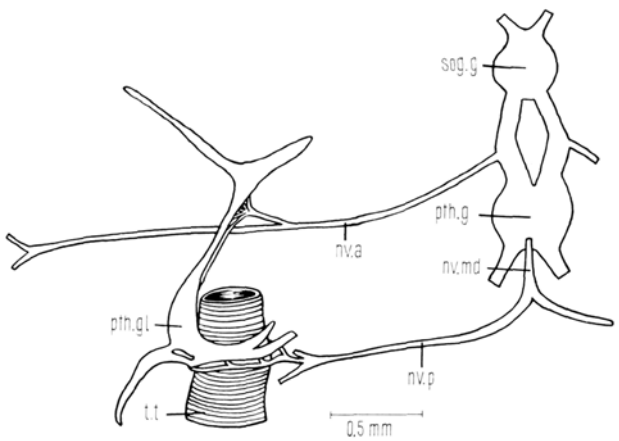

Fig. 1. Camera-lucida diagram showing the innervation of the prothoracic gland in *Papilio demoleus*. nv.a, nv.p, nv.md, anterior, posterior and median nerves respectively; pth.g, prothoracic ganglion; pth. gl, prothoracic gland; sog.g, suboesophageal ganglion; t.t, tracheal trunk.

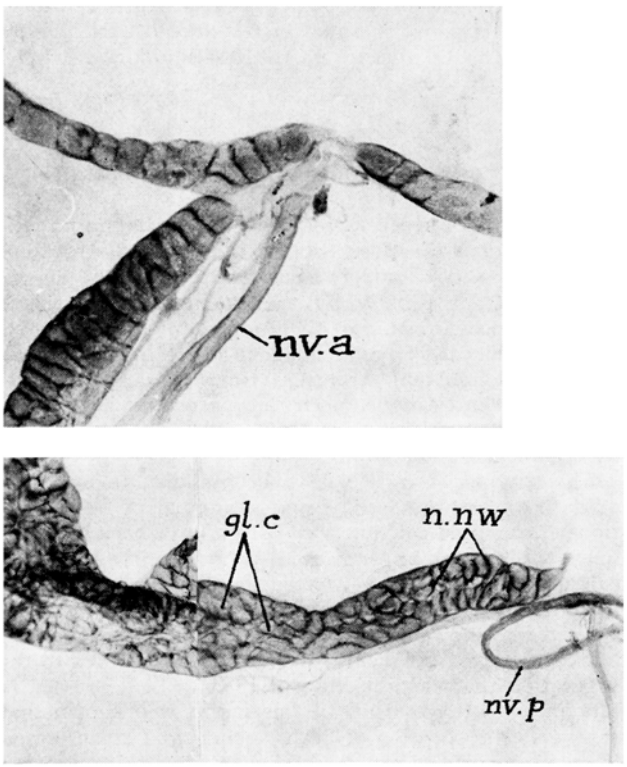

Fig. 2. Photomicrographs of the prothoracic gland stained in leucomethylene blue to show the nerve network. gl.c, gland cells; n.nw, nerve network. Remaining lettering same as in Figure 1.

[1] L. ARVY and M. GABE, C. r. hebd. Séanc. Acad. Sci., Paris *237*, 844 (1953b).
[2] U. S. SRIVASTAVA, Q. Jl. microsc. Sci. *100*, 51 (1959).
[3] C. M. WILLIAMS, Biol. Bull. *94*, 60 (1948).
[4] W. S. HERMAN and L. I. GILBERT, Gen. comp. Endocr. *7*, 275 (1966).
[5] B. STAY and A. GELPERIN, J. Insect Physiol. *12*, 1217 (1966).