in PP (see Figure 4 in BALTZER and CHEN[5]). Hybrid embryos aged 15 h are stereoblastulae, filled with degenerating materials in their cavity. A part of these embryos attempts to gastrulate, though later than PP, but does not develop further. The early abnormality resulting from the elimination process is distinctly reflected in the values of thymidine uptake. Although the values of hybrid embryos at the cleavage stages ($4^1/_2$–6 h) still amount to approximately half of PP, they very soon drop behind and are much lower than those of both parental species at 15 h already: PP about 120, SS 67, and PS 47. Finally at 39–48 h DNA synthesis in the hybrid drops to only about one seventh of that in the maternal controls.

Again there is a certain parallelism between the rate of thymidine incorporation and the nuclear numbers. At 18–20 h there are about 400 nuclei in PS, compared to about 600 in PP and 400 in SS. But the value rises to 517 and 670 in the PS hybrid aged 41–48 h, whereas within the same period it increases to 956 and 1400 in PP and to 669 and 692 in SS (compare CHEN and BALTZER[2], p. 237, and some recent counts).

From their autoradiographic experiments, FICQ and BRACHET[13] concluded that the incorporation of H^3-thymidine into DNA is higher in AA than in PP. A direct comparison between their study and the present one is not possible because, in addition to differences in the techniques used, they investigated mainly the early development while more data are available to us for later stages. Besides the rate of development, which is slower in AA than in PP, other factors like the pool size and the cytoplasmic uptake of the precursor might account for such a discrepancy.

The data presented by FICQ and BRACHET[13] further indicate a considerably higher incorporation of thymidine in PA than in PP for the stages investigated by them. On the other hand, we found a much lower uptake of this labelled precursor in PA than in PP, at least from the mesenchyme blastula stage onwards. Since they disclosed that DNA synthesized by the hybrid is abnormally unstable, it seems possible that the low values of PA determined by us are partly due to loss of some labelled DNA by the extraction procedure.

Another point of interest is that their autoradiographs suggest the elimination of chromatin in the cytoplasm of the PA hybrid. However, in agreement with our observation, no chromatin elimination could be detected by the Feulgen staining. This phenomenon certainly deserves a closer examination.

According to evidence available, protein formation takes place through the template mechanism of RNA which is in turn DNA-dependent. It would be of interest to know to what extent the inhibition of DNA synthesis is directly related to the lethality of the hybrids. During the first 17 h of development, almost all PA hybrids develop normally and do not differ from the PP controls. Thereafter both types of embryos are characterized by an indentation of the vegetative part, indicating the beginning of gastrulation. It is at this critical stage that the development of PA becomes retarded. Even though up to this time PA develops as normally as the maternal controls, its DNA synthesis, as indicated by the incorporation of H^3-thymidine, is already reduced to about half of PP. The real role of DNA of *Arbacia* in the present hybrid combination is unknown. According to MOORE[18], the abnormal development could be a consequence of inexact copies of the genetic material. But, as suggested by BRACHET[19] and BRACHET et al.[20], the possibility that it affects indirectly the embryogenesis through an abnormal synthesis or utilization of RNA is not excluded. Unfortunately, we have no data for still earlier stages. A comparison between this hybrid and the merogonic combination (P)A would be desirable[21].

Zusammenfassung. (1) Es wird die DNS-Synthese der letalen Seeigelbastarde *Paracentrotus* ♀ × *Arbacia* ♂ (PA) und *Paracentrotus* ♀ × *Sphaerechinus* ♂ (PS) mit H^3-Thymidin untersucht. (2) Dem verschiedenen, der Letalität vorausgehenden Entwicklungstypus (PA ohne, PS mit Chromosomenelimination) entspricht ein verschiedener Verlauf der Hemmung der DNS-Synthese. Parallel dazu wird die Vermehrung in der Anzahl der Kerne verglichen.

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[18] J. A. MOORE, Exp. Cell Res., Suppl. *6*, 179 (1958).
[19] J. BRACHET, J. cell. comp. Physiol. *60*, Suppl. 1, 1 (1962).
[20] J. BRACHET, N. BIELIAVSKY, and R. TENCER, Bull. Cl. Sci. Acad. roy. Belg. *48*, 255 (1962).
[21] Acknowledgments: Our previous studies on the biochemistry of sea urchin hybrids as well as the present work were aided by grants from the 'Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung'. We are greatly indebted to the 'Nationalfonds' for generous support and to the authorities of the Zoological Station at Napoli for supply of materials and facilities. We wish also to express our thanks to Miss F. HANIMANN for her valuable help in the course of our experiments.

Agglutinating Antibrain Antibodies in Dogs with Experimental Allergic Encephalomyelitis

Although experimental allergic encephalomyelitis (EAE) has been induced in various animals, it has not always been possible to reveal circulating antibrain antibodies in these animals[1,2]. Antibrain antibodies have been detected in the sera of dogs and monkeys with EAE, without a definite correlation between the occurrence of circulating antibodies and the occurrence of the disease being ascertained[3,4]. Animals have been found which had circulating antibrain antibodies without clinical signs of EAE, and animals that displayed severe signs of EAE without having antibrain antibodies in their sera[1].

[1] B. D. JANKOVIĆ and M. ISVANESKI, Int. Arch. Allergy *23*, 188 (1963).
[2] P. Y. PATERSON, J. Immunol. *78*, 472 (1957).
[3] C. E. LUMSDEN, E. A. KABAT, A. WOLF, and A. E. BEZER, J. exp. Med. *92*, 253 (1950).
[4] L. THOMAS, P. Y. PATERSON, and B. SMITHWICK, J. exp. Med. *92*, 133 (1950).

In a typical experiment we succeeded in finding serum antibrain antibodies in a group of animals, all of which had EAE.

Nineteen dogs were injected subcutaneously with homologous brain homogenate, emulsified in Freund's complete adjuvant, the injections being repeated at 14 day intervals[4]. All the animals showed clinical signs of EAE (weakness, ataxia, paralysis) between the 30th and the 120th day after the first inoculation of encephalitogenic emulsion. The animals with clinical signs were sacrificed. In all these cases, the histopathologic examinations showed characteristic microscopic lesions of EAE. After the first inoculation and until the sacrifice, blood was collected from all the animals at an average interval of 5 to 6 days. The antibrain antibodies were investigated by the tanned hemagglutination technique[5], using as antigens a saline extract of dog brain, as well as a water-soluble fraction extracted from dog brain[6].

In all the animals, circulating antibrain antibodies were demonstrated with varying titers between 1/10 and 1/360, at different time intervals starting on the 7th day after the first inoculation of encephalitogenic emulsion. As some other workers found[4], we also detected antibody titers in the same animal; they varied and at a certain moment they disappeared and subsequently reappeared. About 10 days before the onset of EAE, in almost all animals (except 2 dogs), it was not possible to show serum antibrain antibodies.

Although not all the animals had serum antibrain antibodies on the same day, antibodies have been demonstrated within 30 days in all living animals. The data (Table) indicates that the majority of animals exhibit clinical signs of EAE 90 days after the first inoculation with encephalitogenic emulsion. In the present experiments we have found no animals with serum antibrain antibodies without EAE, while animals with EAE did not have antibrain antibodies. As all animals presented circulating antibrain antibodies at various time intervals, and all of them fell sick with EAE, it seems that a close relationship might exist between the occurrence of serum antibrain antibodies and EAE. However, the fact that circulating antibrain antibodies were not detected at the onset of the disease, makes this correlation rather unreliable.

It has been shown[7] that the complement-fixing antibrain antibodies play a protective role and exert a suppressive influence on the development of EAE when passively administered to animals actively sensitized to nervous tissue. It may be that a similar part is played also by antibrain antibodies as demonstrated by the passive hemagglutination reaction.

Animals with circulating antibrain antibodies at various time intervals

Time interval (in days)	No. of dogs with serum antibrain antibodies	No. of dogs with clinical signs of EAE[b]
0–30	19 (100%)[a]	1
30–60	18 (100%)	–
60–90	18 (100%)	6
90–120	12 (100%)	12
Total animals with EAE		19

[a] Figures in parentheses show the percentage of animals with antibrain antibodies out of the total number of surviving animals.
[b] Animals which were sacrificed when exhibiting clinical signs of EAE.

Résumé. Chez 19 chiens présentant des symptomes de EAE, on a trouvé des anticorps sériques anticerveaux pendant divers laps de temps.

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N. Rotaru, and A. Vladutiu

Department of Pathologic Physiology, Institute of Medicine, Bucharest (Roumania), November 13, 1964.

[5] E. V. Boyden, J. exp. Med. *93*, 107 (1951).
[6] R. F. Kibler and A. E. Barnes, J. exp. Med. *116*, 807 (1962).
[7] P. Y. Paterson and S. M. Harwin, J. exp. Med. *117*, 755 (1963).

Cancerostatische Proteinkomponenten aus *Viscum album*

Frühere Untersuchungen über cancerostatische Inhaltsstoffe von *Viscum album* L. zeigten die höchste Aktivität in der Gesamtproteinfraktion des Preßsaftes[1]. Unseres Wissens ist dies die erste proteinartige Substanz, die eine hohe Hemmwirkung an experimentellen Tumoren zeigt[2].

Später berichteten Winterfeld et al.[3] über die Anreicherung einer peptidartigen Substanz aus *V. album*. Die tumorhemmende Wirkung der dabei isolierten Endfraktion ist jedoch lediglich mit derjenigen unseres Ausgangsextraktes vergleichbar. Watanabe et al.[4] gelang es dagegen vor kurzem, ein Protein aus *Flammulina velutipes* zu isolieren, dessen cancerostatische Wirkung bereits in die Grössenordnung unserer Stufe [3] (Figur 1) fällt. Wir wollen mit diesem Beitrag den vorläufigen Stand unserer eigenen Arbeiten mitteilen. Der in Figur 1 schematisierte Aufarbeitungsgang[5] erlaubt es, die biologische Aktivität des schonend gewonnenen Pflanzenextraktes[6], einer ED_{50} von 0,3 mg/kg, auf das 150fache anzureichern (Tabelle).

[1] O. S. Selawry, F. Vester, W. Mai und M. Schwartz, Hoppe Seyler's Z. *324*, 262 (1961).
[2] Hier sei auf eine Arbeit von W. B. Coley hingewiesen (Trans. Am. Surg. Ass. *12*, 183 (1894)), der bereits Ende des vorigen Jahrhunderts quasi als biologische Alternative zu den bislang bekannten synthetischen Zellgiften die Anwendung eines toxinhaltigen Extraktes von *Bacillus prodigiosus* zur Tumorbekämpfung empfahl.
[3] K. Winterfeld, O. S. Selawry, M. Grunthal und M. Schwartz, Arzneimittelforschung *13*, 29 (1963).
[4] Y. Watanabe, K. Nakanishii, N. Komatsu, T. Sakabe und H. Terakawa, Bull. chem. Soc. Japan *37*, 747 (1964).
[5] F. Vester, Patentschrift, Belg. 646095 v. 4.4.1963.
[6] F. Vester und W. Mai, Hoppe Seyler's Z. *322*, 273 (1960).