dation. This article is contribution No. 2143 from the Gates and Crellin Laboratories of Chemistry, California Institute of Technology.

5. D. H. Campbell *et al.*, *Science* 119, 289 (1954).
6. A. G. Gornall *et al.*, *J. Biol. Chem.* 177, 751 (1949).
7. M. H. Loveless *et al.*, *Quart. Rev. Allergy and Appl. Immunol.* 9, 81 (1955).
8. W. J. Kuhns, *J. Immunol.* 75, 105 (1955).
9. We have also found that components that can induce skin-sensitizing activity can be separated from precipitating antibody by ammonium sulfate fractionation. A discussion of these studies is in preparation.
10. S. J. Singer and D. H. Campbell, *J. Am. Chem. Soc.* 77, 3504 (1955).

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"Nonviral" Tumors Produced in Turkeys by Rous Sarcoma Virus

The marked variability in the quantity of virus present in tumors of known viral etiology is well known and has been emphasized in recent reviews (*1*). In the case of the Rous sarcoma, Bryan *et al.* (*2*) have shown that the amount of virus extractable from the tumor is related to the initiating dose of virus. Indeed, when dilutions of Rous sarcoma virus were employed that produced tumors in less than half of the chickens, about 24 percent of such tumors yielded no recoverable virus at all. Duran-Reynals (*3*) found that tumors were produced in turkeys by Rous sarcoma virus and that these tumors could be transferred by means of cell suspensions or extracts through four serial passages in young turkeys, although some loss in potency was observed. The virus retained its infectivity for chickens throughout its passage in turkeys. Harris (*4*) observed that although very young turkeys were susceptible to this virus, they became resistant after 3 weeks of age. Intravenous inoculation of chicken blood into newly hatched turkeys or embryonated turkey eggs markedly decreased the development of resistance.

Sarcomas produced in turkeys with as much as 10,000 ED_{50} of chicken tumor virus yielded little or no extractable virus (Table 1), despite the fact that the dilution end-point for tumor production by chicken tumor virus was identical in chicks and turkeys. In these experiments, 0.2-ml amounts of serial decimal dilutions of standard Rous sarcoma virus that had been prepared from chicken tumor tissue by differential centrifugation (*5*) were inoculated subcutaneously into the wing web of groups of 10 to 20 chicks and turkeys, respectively. Unsexed white leghorn chicks and Beltsville white turkey poults 3 to 6 days of age were used, and the birds were examined daily for 4 to 6 weeks. As anticipated (*3*) the sarcomas produced in turkeys were grossly and histologically different from those produced in chickens. These tumors developed rapidly but, once they were established, they grew slowly, and metastases were almost invariably found in the liver when large amounts of virus were used. Two to 4 weeks after inoculation of virus, three turkeys from each dilution group were killed, and their tumors were stored at −70°C in glass-sealed ampoules. Later, each tumor was thawed and ground in a mortar with sand, and sufficient saline was added to make a 10-percent suspension by weight. Each suspension was clarified by centrifugation and inoculated subcutaneously into groups of 11 chicks each, which were examined daily for 4 weeks for the presence of tumors. It is clear that chicks and turkey poults were equally susceptible to Rous sarcoma virus but that the virus was present in appreciable quantities only in turkey tumors produced by large amounts of virus.

Table 1. Production of "nonviral" tumors in turkeys by virus obtained from chicken tumor tissue.

Titration of Rous sarcoma virus					Inoculation of extracts of individual turkey tumors into chicks		
Virus diluted: (log)	Chicks		Turkeys				
	Response*	Latent period† (day)	Response*	Latent period† (day)	Tumor	Response*	Latent period† (day)
					a	11/11	5.0
−1	19/20	4.5	15/15	2.4	b	11/11	5.2
					c	11/11	5.3
					d	0/11	
−3	19/20	5.3	15/15	3.8	e	0/11	
					f	8/11	7.0
					g	0/10	
−5	20/20	6.4	15/15	5.7	h	0/10	
					i	2/10	
					j	2/11	
−6	17/20	8.2	14/15	6.6	k	4/11	> 15.0
					l	10/11	10.0
					m	0/11	
−7	2/10		7/15		n	0/11	
					o	4/11	> 15.0
−8	1/10		2/15		p	0/11	
−9			0/15				

* No. developing tumors/total No. inoculated.
† Latent period is the time in days required to produce tumors in 50 percent of chicks inoculated (estimated graphically using probability paper; $y = 100/\text{No. of days}$).

Table 2. Serial passage of Rous sarcoma virus in turkey poults.

Passage number	Response	Latent period (day)
1	22/22	5.3
2	33/33	8.1
3	28/28	8.6
4	3/19	
5	0/25	

Table 2 shows that serial passage of Rous sarcoma virus in turkeys was associated with a progressive loss in potency with each passage until the fourth passage, when extracts of such tumors did not produce tumors in turkeys. Standard Rous sarcoma virus derived from chicken tumor tissue was used to initiate the first passage in turkeys. Ten-percent tissue extracts for the subsequent passages were prepared from pools of three turkey tumors each. The methods employed were the same as those described in the previous paragraph, except that the tumor tissue extracts were subjected to additional centrifugation (*2*) to remove any intact cells that might have escaped disintegration during freezing, thawing, and grinding. Extracts of tumors from the third passage produced tumors in only 15.8 percent of the turkeys, and these tumors contained no demonstrable virus.

In addition, 10-percent tissue extracts were prepared from two individual tumors and one pool of two tumors from the third serial turkey passage, and each of these was inoculated subcutaneously into groups of ten or more chicks each. Two of these extracts failed to produce tumors in chicks, but one extract contained a quantity of virus equal in potency to standard Rous sarcoma virus, indicating that such tumors can occasionally contain appreciable amounts of recoverable virus.

It is possible that the development of natural or acquired resistance in the turkey (*4*) and the effect of the initiating dose on the yield of extractable virus (*2*) both contribute to the low viral content of turkey tumors. In any event, the frequent production by Rous sarcoma virus of noninfective tumors in turkeys (*6*) resembles the so-called "masked" virus

described by Shope (7) for papilloma virus in domestic rabbits and pseudorabies virus in cattle, as well as the neuropathic effect produced in mice by high dilutions of certain strains of Newcastle disease virus (8).

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References and Notes

1. J. Beard, D. G. Sharp, E. A. Eckert, *Advances in Virus Research* 3, 149 (1955); J. W. Beard, *Cancer Research* 16, 279 (1956).
2. W. R. Bryan, D. Calman, J. B. Moloney, *J. Natl. Cancer Inst.* 16, 317 (1955).
3. F. Duran-Reynals, *Cancer Research* 3, 569 (1943).
4. R. J. C. Harris, *Proc. Roy. Soc. (London)* B146, 59 (1956).
5. W. R. Bryan, J. B. Moloney, D. Calnan, *J. Natl. Cancer Inst.* 15, 315 (1954).
6. This investigation was supported by a grant-in-aid from the American Cancer Society and by funds provided by the Rutgers Research and Endowment Foundation.
7. R. E. Shope, in *Viruses*, M. Delbruck, Ed. (California Institute of Technology, Pasadena, 1950).
8. V. Groupé and R. M. Dougherty, *J. Immunol.* 76, 130 (1956).

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Biological Concentration by Killer Clams of Cobalt-60 from Radioactive Fallout

After the March 1954 nuclear detonation in the Pacific Ocean, a number of the northern Marshall Islands were contaminated with radioactive fallout (1). Since that time, our laboratory has made periodic surveys of the area to evaluate the residual contamination in plants, marine and land animals, soil, and water (2). Among the specimens collected at two years postdetonation were two "killer" clams (*Tridacna gigas*) that were recovered from the shores of Rongelap Island (3).

The soft tissue of the clams was prepared for analysis by the dry-ash method at 500°C and dissolved in dilute acid. Measurements of gross activity on aliquots of the resulting solution revealed the presence of readily detectable amounts of both beta and gamma radiations. As an aid to identification, the samples were subjected to gamma spectrum analysis in a single-channel analyzer. Gamma photons of energies 1.17 and 1.33 Mev which are identical with those of Co^{60} were observed. Confirmation of the presence of this nuclide was sought by chemical separation and by additional radiation characterization.

Table 1. Cobalt-60 and gross gamma activity in killer clams.

Specimen	Wet Wt. (g)	Gamma Activity		Co^{60}* (disintegration/min)†
		Gross count/min	Co^{60}* (count/min)	
A	1800	142,700	90,300	210,000
B	882	356,700	303,000	705,000

* Corrected for recovery.
† The disintegrations per minute were determined by comparison with a Co^{60} source obtained from the National Bureau of Standards.

To establish the reliability of the analytic procedure, a preliminary experiment was devised for evaluating the exchange of Co^{60} with cobalt carrier and the decontamination efficiency from other radioactive elements. Cobalt-60 tracer and cobalt carrier ($CoCl_2$) were added to a 1-month-old solution of mixed fission products. A control was maintained in which the addition of Co^{60} was omitted. The solution was twice scavenged with ferric hydroxide, using ammonium hydroxide for alkalinization and complexation. The cobalt was then precipitated with α-nitroso-β-naphthol (4). Recovery was determined by the colorimetric nitroso-R salt method (5).

The reliability of the analytic procedure was evident from the results of the preliminary experiment. Cobalt was decontaminated from mixed fission products with 99 percent efficiency, and exchange was complete with a 20-percent yield of both carrier and activity.

This analytic procedure was applied to the specimens. The results of analysis are given in Table 1. For the purpose of comparison, the gross gamma count is also included. The data clearly indicate that the greater fraction of the gamma activity was attributable to Co^{60}. In specimens A and B, the activity contributed by this nuclide was 63 and 85 percent of the gross gamma activity, respectively.

To establish the identity of the isolated activity unequivocally, the radiations were characterized by aluminum and beryllium absorption curves and by analysis of the gamma spectra. In each case the characteristics were identical with those of an authentic sample of Co^{60}.

The appearance of readily measurable quantities of Co^{60} in the killer clam is noteworthy from two aspects. First, Co^{60} is not a component of fission products. It is therefore assumed that this nuclide was induced from an environmental precursor by the neutron flux accompanying the nuclear detonation. A possible precursor is natural Co^{59}, which, when bombarded by neutrons, undergoes the typical (n, γ) reaction to form Co^{60}. More importantly, this radioelement was not detected in the numerous fallout-exposed materials analyzed at one year postdetonation (1). Presumably the induced activity was present only in trace amounts. The accumulation of Co^{60} from an environment which for all intents and purposes was infinitely dilute points to the enormous concentrating capacity of the killer clam. Experiments are currently underway to establish whether this property is common to all bivalves.

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References and Notes

1. R. W. Rinehart *et al.*, *U.S. Naval Radiol. Defense Lab. Rept. No. USNRDL-454* (2 Sept. 1955).
2. The work reported was done under the sponsorship of the U.S. Atomic Energy Commission and the Bureau of Ships, project No. NS-081-001.
3. We extend appreciation to J. K. Gong of our Laboratory for collecting the killer clams.
4. W. F. Hillebrand *et al.*, *Applied Inorganic Analysis* (Wiley, New York, ed. 2, 1953).
5. J. N. Pascual, W. H. Shipman, W. Simon, *Anal. Chem.* 25, 1830 (1953).

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Observations on a Fast-Moving Protein in Avian Malarial Serum

The alterations occurring in the electrophoretic patterns of the serum proteins of men and animals infected with the malaria parasite have been extensively investigated by the now classical moving-boundary method of Tiselius. The results of these studies have been summarized by Stauber (1) in a recent review of the application of electrophoretic techniques in the field of parasitic diseases. In general, no qualitative changes have been proved, but decreased albumin and increased globulin, particularly alpha-2 and gamma globulin, have been shown to occur. This preliminary report describes a marked qualitative change, which was found by utilizing filter-paper electrophoresis, that occurs in the serum protein patterns of pigeons infected with the 1Pl-1 strain of *Plasmodium relictum* (2)—namely, the appearance of a new component possessing an electrophoretic mobility greater than that of albumin.

Paper electrophoresis offers an important advantage over the Tiselius method because it requires an extremely small sample for analysis. It is thus ideally suited to the serial examination of the blood proteins of small laboratory animals without material interference with the usual course of an induced infection. A modification of the horizontal open-strip method of Grassmann and Hannig (3) was employed in these studies. Four-hundredths of a milliliter of serum was applied by micropipet to Whatman No. 1 filter paper strips (35 by 3.75 cm) immersed in a Veronal-acetate buffer of *p*H 8.6 and ionic strength 0.1. A constant current of 0.5 ma per centimeter of paper width was applied for a period of 19 hours at room temperature (23 to