[1] See Dunford, N., and J. T. Schwartz, *J. Rational Mech. Anal.*, **5**, 129–178 (1956). In what follows, $\mathfrak{M}$ will denote a general measure space with points $x, y, \ldots$, and measure dx.

[2] The case $p = 2$ of the theorem is due to D. L. Burkholder and Y. S. Chow, *Proc. Amer. Math. Soc.*, **12**, 490–495 (1961). This article was brought to our attention by G. C. Rota. We had independently obtained the general theorem in 1958, but did not publish it. Recently Rota has obtained another approach to the theorem which will appear elsewhere. See also C. Herz, *Proc. Amer. Math. Soc.*, **12**, 229–233 (1961).

[3] These notions were used in the article of E. M. Stein and G. Weiss, *Tohoku Math. J.*, **9**, 318–339 (1957), especially section 3.

[4] Stein, E. M., *Trans. Amer. Math. Soc.*, **83**, 482–492 (1956).

[5] See the argument in R. E. Paley, *Proc. London Math. Soc.*, **31**, 289–300 (1930).

[6] In the cases mentioned, the kernels are nonnegative. See G. N. Watson, *J. London Math. Soc.*, **8**, 189–192, 194–199, 289–292 (1933).

[7] McKean, H. P., *Trans. Amer. Math. Soc.*, **82**, 519–548 (1956).

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*HEAT-RESISTANCE OF THE TUMORIGENIC NUCLEIC ACID OF SHOPE PAPILLOMATOSIS**

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Previous studies from this laboratory have demonstrated that nucleic acid-containing extracts of papillomatous tissue from wild cottontail rabbits can induce tumor in domestic rabbits.[1–3] The biologically active factor in the extract is known to be DNA in nature. Recently Watson and Littlefield have reported that the nucleic acid of Shope papilloma virus is a double-helical DNA and has a melting point (T_m) of 89.5°C.[4] Since the Shope papilloma virus is known to be inactivated by much lower temperature (70°C, 30 min.),[5] the following questions occurred to us: (1) is heat-inactivation of this virus the result of denaturation of the viral protein rather than nucleic acid, and if so, (2) how would exposure of the "tumorigenic DNA" to a temperature above the melting point affect its biological activity? To answer these questions we investigated the biological activity of nucleic acid extracted from Shope papilloma virus completely inactivated by heat and also studied the tumorigenic activity of the nucleic acid-containing extract after exposure to 100°C.

Materials and Methods.—The starting materials for the virus and the nucleic acid extracts were the glycerinated warts of wild cottontail rabbits originally collected in Kansas (Earl Johnson Farm). A part of the material from the same source was kindly provided by Dr. R. E. Shope of the Rockefeller Institute. The virus preparation was a 10 per cent (v/v) extract of cottontail papilloma tissue in phosphate-buffered saline[6] without magnesium and calcium (PBS). Five ml of virus preparation was exposed to a temperature of 70°C in a heated water bath for a desired period of time. After the thermal treatment, 1 ml was saved for a test to detect surviving intact virus. The remaining 4 ml was subjected to phenol extraction with an equal volume of 80 per cent phenol in PBS. The procedure has been described in detail in the preceding paper.[1] To precipitate the nucleic acids, 2 volumes of cold ethanol were added to the final aqueous solution of the extract and the precipitate was taken up in 1 ml of PBS.

The tumorigenic nucleic acid extracts used for the heating experiments were prepared by our standard procedure[1] and had a DNA content of approximately 200 to 250 μg/ml as assayed by the modified diphenylamine method of Burton.[7] Two kinds of preparations,[3] "undiluted" and

alcohol "precipitated," were employed. The extracts (0.5 ml) were placed in a rubber-capped glass tube and immersed in a boiling water bath for a desired period of time. After heating, the material was either slow or fast cooled according to the criteria introduced by Doty and Marmur and their group.[8, 9] For fast cooling, the extracts were placed immediately in an ice-cooled water bath. The slow cooling was carried out by transferring the extracts to a bath with 6 liters of water preheated to 90°C. The heater was turned off and the bath was left at room temperature to cool down gradually to 50°C. In a typical experiment, the drop of the temperature took the following time course: 90°–0 min, 80°–20 min, 70°–60 min, 60°–110 min, and 50°–170 min.

The tumorigenic activity of the virus and the nucleic acid extracts was tested by inoculating 0.1 ml of the test preparation into clipped and shaved areas of skin of domestic rabbits (New Zealand White Breed) by the intradermal injection and puncture method.[3]

Experimental.—Recovery of tumorigenic nucleic acid extracts from the heat-inactivated Shope papilloma virus: From the data listed in Table 1, it has been shown that the nucleic acid preparations extracted from the Shope papilloma virus completely inactivated by heating at 70°C did indeed retain their tumorigenic activity.

TABLE 1

TUMORIGENIC ACTIVITY OF THE WHOLE VIRUS AND ITS NUCLEIC ACID EXTRACTS AFTER HEATING SHOPE PAPILLOMA VIRUS PREPARATION AT 70°C

Time of exposure (min)	Preparation	No. tumors per no. inoculation sites	Per cent of take	Average incubation period (days)
0*	Whole virus	6/6	100	13
"	Nucleic acid extract	11/18	62	19
30	Whole virus	±1/16†	0	..
80	" "	0/16	0	..
180	" "	0/16	0	..
80	Nucleic acid extract	9/16	56	22
180	" " "	5/10	50	21
360	" " "	4/18	27	22

* Unheated control.
† A scanty papilloma was seen after 60 days.

The exposure time in these experiments was 2.5 to 12 times longer than the minimal time required for total inactivation of intact virus. In addition to other criteria,[3] these findings provide a temperature differentiation between the tumorigenic activity of Shope papilloma virus and of nucleic acid extracts.

Resistance of Tumorigenic Activity of Cottontail Papilloma Nucleic Acid to Heat at 100°C.—A greater thermal stability of the nucleic acid moiety over the whole virus itself has been demonstrated in RNA viruses.[10, 11] The results of the present experiment have shown that the same general picture is also true of Shope papilloma virus. As shown in Table 2, nucleic acid extracts treated at 100°C for 5 to 30 min produced tumor growth at the site of inoculation. Preparations heated for 1 hr failed to show the growth of tumor after 60 days of observation. Little difference was encountered between fast and slow cooling. Although all preparations heated up to 30 min yielded definite growth of the tumor, prolongation of incubation period was observed as the heating time increased. This suggests a partial loss of infectivity under these conditions.

Discussion.—The tumorigenic nucleic acids employed in the heating experiment were about average preparations among our extracts in their capacity to induce tumor in domestic rabbits. Despite the high DNA content of 200 to 250 μg/ml, which is not of course representative of the content of tumorigenic DNA, the infectious level of DNA in the extract was only about 2 to 3 times that of the minimal infective dose (ID_{100} = approximately 80–100 μg/ml). At this level, therefore, it

TABLE 2

TUMORIGENIC ACTIVITY OF COTTONTAIL PAPILLOMA NUCLEIC ACID PREPARATIONS AFTER HEATING AT 100°C

Experiment	Time of exposure (min)	Preparation	DNA content (μg/ml)	Mode of cooling	No. tumors per no. inoculation sites	Average incubation period (days)
1	0*	Undiluted	207	...	4/4	15.2
	5	Undiluted	"	Slow	4/4	18.2
				Fast	4/4	17.0
	10	Undiluted	"	Slow	4/4	19.5
				Fast	4/4	18.0
2	0*	Precipitated	235	...	2/2	10.0
	10	Precipitated	"	Slow	2/2	14.5
				Fast	2/2	15.5
	20	Precipitated	"	Slow	2/2	15.5
				Fast	2/2	17.5
	30	Precipitated	"	Slow	2/2	19.5
				Fast	2/2	19.0
3	0*	Undiluted	250	...	2/2	13.5
	20	Undiluted	"	Slow	2/2	16.0
				Fast	2/2	15.5
	30	Undiluted	"	Slow	2/2	18.5
				Fast	2/2	22.5
	60	Undiluted	"	Slow	0/2	...
				Fast	0/2	...

* Unheated control.

seems unlikely that the thermal stability demonstrated above is due to a great excess of active agent in the solution to start with. However, the 100 per cent inactivation time will vary with the concentration of tumorigenic DNA in the extract.

Since our DNA preparation still contains an appreciable amount of orcinol (Bial) reacting substance and protein[3] in addition to cellular nonviral DNA, it is not possible at the present time to discuss the events taking place at the molecular level in heating and cooling of extracts. However, despite the presence of impurities it is hard to believe that the DNA in the extract can remain "unmelted" after treatment at 100°C for 30 min when its T_m is only about 90°C.[4] Assuming that melting does occur, it must be considered that either single-stranded DNA is able to initiate neoplastic growth, or else *renaturation*[8] has occurred. The UV absorption studies of Shope papilloma virus DNA by Watson and Littlefield suggested that there is renaturation of the heated Shope virus DNA. Further studies with DNA preparations of greater homogeneity may help to decide between these alternatives.

Summary.—Shope papilloma virus preparations which had been completely inactivated by exposure to heat at 70°C were shown to yield tumorigenic nucleic acid extracts by the phenolic deproteinization procedure. The tumor-inducing capacity of the nucleic acid preparations obtained from papillomatous tissue of cottontail rabbits was demonstrated to survive treatment at 100°C for 30 min.

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*THE DISTRIBUTION IN THE TISSUES AND THE DEVELOPMENT OF H-2 ANTIGENS OF THE MOUSE**,†

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The study of histocompatibility antigens of the mouse by hemagglutination techniques has disclosed a striking complexity of the antigenic products of the *H-2* locus and a rather disappointing simplicity, or the nonexistence in red cells of agglutinogens determined by other *H-loci*.[1] The recognition of histocompatibility antigens through hemagglutination has the further inconvenience of limiting the search only to those that are present simultaneously in the fixed tissues and in the red cells. In spite of these limitations, the importance of cell antigens ("H-antigens") for tissue compatibility in transplantation,[2, 3] tolerance phenomena, runt disease,[4] enhanced growth of tumor homotransplants,[3] and the biological protection of lethally irradiated animals,[5, 6] points to the need of more knowledge about these antigens.

This report is an effort in that direction and refers to a study of the relationships between genes and antigens as observed in extremely favorable material, the histocompatibility-2 (*H-2*) genes and antigens of the mouse.

H-2 is a complex system of tissue- and blood-cell-antigens which are inherited as one block[1, 8, 9] in much the same way as the Rh antigens[10] or some of the complex antigenic systems of cattle.[11] The advantage of this material is shown by the existence of a number of inbred strains whose genetic constitutions are well known, and especially by the development by G. D. Snell of a series of stocks that differ at this particular locus but are otherwise isogenic—the so-called isogenic resistant, or IR strains.[12] This makes it possible to obtain all genotypes needed, which is difficult or impossible in other species of mammals.

The *H-2* system of antigens is determined by a series of alleles, each one of which is characterized by a sort of antigenic code of the type shown in Table 1, where only 7 out of 18 or more alleles and phenotypes known are indicated. The antigens listed are those already found both in the red cells and tissues. It is very probable that there are other antigens of this system, but they are not detected with hemag-