of tallow to similar diets containing untreated pearled barley gave chick growth and feed efficiency almost comparable with a diet containing corn. The possibility that an inhibiting substance was destroyed by the treatment cannot be eliminated, since appropriate experiments have not been conducted.

Summary. The results obtained in 2 independent experiments demonstrated that a simple water treatment of pearled barley markedly improved the nutritional value of this cereal grain. Treated barley was equal to corn for chick growth. The diet containing treated pearled barley gave significantly better ($P<.01$) feed efficiency than the diet containing corn or untreated pearled barley.

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Immunogenicity of Poliomyelitis Vaccine Prepared with Ultraviolet Irradiation and Mild Heat.* (23184)

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In previous reports(1,2) it was demonstrated that poliomyelitis vaccines produced by inactivation with ultraviolet irradiation alone or in combination with heat were safe and effective when used in human volunteers. In this paper, experiments showing effects of varying intensities of ultraviolet irradiation with and without subsequent exposure to mild heat on inactivation of poliomyelitis virus and on antigenicity of the resulting vaccine in animals, are presented.

Material and methods. The vaccines were prepared from poliomyelitis virus propagated in monkey kidney tissue culture. Some preliminary work was done with vaccines made from virus grown in our laboratories; however, the bulk of the virus preparations used to prepare vaccines for this study was supplied by Parke, Davis and Co. and contained Mahoney (Type I), MEF-1 (Type II), and Saukett (Type III) strains. The material, except in one experiment, was irradiated as trivalent pools after preliminary studies had demonstrated that all 3 types of virus were essentially equally susceptible to ultraviolet irradiation, as shown in Table I. Just prior to initial irradiation the virus suspensions were filtered through a series of sintered glass filters (coarse, medium-fine and ultra-fine). In cases where 2 irradiations were employed in series (see below), an intermediate fine sintered glass filter was used. The irradiation was carried out in centrifugal filmers described in detail elsewhere†(3). Two filmers were connected in series in such a manner that samples could be taken after the virus suspensions had passed through one or both of them. The quantity of ultraviolet energy absorbed/ml of virus suspension was varied by passing the respective materials through the machine(s) at different rates. While exposure time is relatively constant, the net biological effect produced by irradiation is dependent upon a number of factors, discussed elsewhere†(1,3). At a flow rate of 200 ml/minute in the particular machine used, the film thickness is about 18 μ. An increase in flow rate results in an increase in film thickness, thereby reducing the quantity of energy absorbed per unit volume irradi-

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† To be published.

TABLE I. Titers of Poliomyelitis Virus Strains before and after Ultraviolet Irradiation.*

Virus strain irradiated	Titer† before irradiation	Titer after irradiation‡ at 25 watts	20 watts	15 watts	10 watts
Type I (Brunhilde)	6.8	1.2	1.1	3.1	4.4
I (Parker)	6.7	.6	.9	2.6	4.1
II (MEF)	6.8	1.0	1.5	3.5	4.4
III (Saukett)	7.0	1.1	1.5	3.0	3.7

* Table based on several experiments with each strain of virus, using 0.5 ml inoculation per tube of tissue culture, 10 tubes per level, log dilution series.
† $TCID_{50}$ (0.5 ml inocula) in monkey kidney tissue tube cultures.
‡ Irradiation in Centrifilmer at rate of 600 ml/min.

TABLE II. Irradiation Titration-Poliomyelitis Virus in 199 Fluid.

Initial titer	Irradiation technic: ml/min.	Watts	No. machines	Post irradiation titration: Undil.	Tubes* 10^{-1}	10^{-2}	10^{-3}	Bottles†
7.1	200	20	2	0/30				0/5
6.0	"	"	2	0/20				0/3
7.9	400	"	2					0/5
7.2	600	"	2	0/10				3/3
6.0	"	15	2	0/10				3/3
6.1	800	20	2	0/10				2/5
6.2	1,000	"	2	2/10	0/10			1/1
7.9	400	"	1	3/ 5	2/10	0/10		
6.1	800	"	1	10/10	7/10	1/10	0/10	
7.2	600	"	1	10/10	10/10	4/10		
6.2	1,000	"	1	5/ 5	5/ 5	3/ 5	0/ 5	
6.0	600	15	1	10/10	5/ 5	2/ 5	1/ 5	

* 0.5 ml inocula/tube. † 50 ml inocula/32 oz bottle.

ated. Flow rates of from 200 to 1,000 ml/minute were employed and the virus suspension was passed through either 1 or 2 machines at these rates. The titers of the resulting irradiated suspensions are shown in Table II. In most studies here reported, 50 ml inocula/bottle were used in the safety tests. Subcultures were made from each bottle to at least 10 tubes of tissue culture after the bottles had been incubated 7 and 14 days, respectively. Since no chemicals were added, it was not necessary to dialyze the irradiated samples prior to performing safety tests. Potency tests of vaccines were made in mice and monkeys by the methods described in "Minimum Requirements: Poliomyelitis Vaccine" —1st Revision, April 12, 1955, by the Nat. Inst. H. Antibody titrations of the animal sera were made in monkey kidney tissue cultures, using the roller tube technic(4) or the metabolic inhibition procedure(5). Our own standard reference serum was used in most of these titrations. Titers are expressed in terms of the 50% end point of serum dilution in all cases.

Results. Table III gives the results of potency tests in mice or monkeys and safety tests on lots of vaccines prepared by irradiation alone. The mouse potency results shown in this Table and those in Table IV are the titers in mice which had received the undiluted vaccine. The monkey potency results are the arithmetic means of the titers of individual monkey serums.

It would appear from the results shown in Table III that the virus suspensions exposed at the rate of 200 ml/minute may have been over-irradiated to a point where antigenicity was impaired. However, increasing the flow rate in each of 2 machines to 400 ml/minute did not appreciably improve potency. With flow rates of 600 ml/minute or higher residual live virus was found when 150-250 ml amounts of vaccine were tested in tissue culture regardless of the wattage employed.

It was then decided to try a combination of irradiation and mild heat to produce inactivation. Table IV gives the results of safety and potency tests of experiments in which combinations of ultraviolet energy, as established

TABLE III. Immunogenicity of Vaccine Inactivated by Ultraviolet Irradiation Alone.

Exp. No.	Trivalent titer, pre-irrad.	Flow rate, ml/min.	Exposure to ultraviolet, watts*	Post irrad. TC-titer Tubes§	Bottles‖	Mouse potency types I	II	III	Monkey potency types I	II	III
1	7.1	200	20–20	0/30	0/5	8	8	256	2	19	3
Control†						4	8	8	0	0	0
5	6.0–6.7‡	"	"	0/20	0/3	16	16	4			
Control						8	8	8			
7	7.0	"	"	"	0/2	4	4	4			
Control						8	8	8			
11	7.9	400	"		0/5	4	64	4	18	45	5
Control						4	4	16			
12	7.9	600	"	0/10	3/3						

* 20–20 signifies that material was subjected to 2 sequential irradiations, with 20 watts of incident energy used in each irradiation.

† Undiluted formalinized control vaccine, supplied by Parke, Davis and Co.: Used in all experiments.

‡ Range of monovalent titers.

§ Tissue culture 0.5 ml/tube.

‖ " " 25-50 ml/bottle.

by flow rates through one or two filmers, are followed by holding the suspensions at 37-40°C for 3 to 20 days. The potency data do not allow an accurate comparison between the several experimental lots because the pre-irradiated suspensions were not identical in all essential respects, especially in titer. However, the results would suggest that the antigenic potential of the vaccines produced by inactivation with ultraviolet and heat compare favorably with the formalin control vaccine.

Virus suspensions which had been inactivated by exposure to irradiation and mild heat for short periods of time, such as 5 days at 37°C were tested for "reactivation" by exposure to mild heat for additional periods of 3 to 5 days. Reactivation did not occur under our conditions; indeed exposure to heat acts as an inactivating agent rather than as a reactivating process. It was also found that exposure to mild heat for as long as 5 days after active virus had disappeared, did not cause a significant reduction in antigenicity. This indicates that a margin of safety of several days' duration can be employed in the inactivation process without impairing antigenicity. There is evidence(6) that mild heat alone does not produce a killed poliomyelitis virus vaccine with satisfactory antigenicity. Apparently, marginal lethal levels of ultraviolet may leave some virus particles viable but injured and incapable of survival if held at temperatures of 37° to 45°C for a few days. On the other hand, virus particles remaining viable after irradiation are inactivated very slowly if placed at 5°C.

Three of the lots of vaccines were retested in animals after storage at 4°C. The results (Table V) indicate only slight antigenic deterioration in vaccines TV8 and TV9 after storage for 6 and 7 months, respectively. Type 1 and 3 components of vaccine TV7 seemed to have deteriorated somewhat more during a storage period of 10 months but there was still a fair amount of antigenicity present.

Discussion. The results presented give capacity of ultraviolet irradiation to destroy cytopathogenic capacity of polio virus under certain experimental conditions. It was found that, if sufficient ultraviolet energy was used to destroy completely the cytopathogenicity of the virus for monkey kidney cells, the antigenic potential was reduced. However, as was shown previously(1), such vaccines still retain the capacity to induce an immune response in humans.

Since the safety tests used to establish the non-cytopathogenicity of inactivated polio virus have been made more critical(7), it appeared reasonable to attempt to use combinations of inactivation procedures. Inactivation of viruses by means of ultraviolet irradiation alone is a physical method and, logically, the

TABLE IV. Immunogenicity of Vaccine Inactivated by Ultraviolet Irradiation and Mild Heat.

Exp. No.	Trivalent titer, pre-irrad.	Flow rate, ml/min.	Exposure to ultraviolet, watts*	Days of mild heat 37°C	Days of mild heat 40°C	Safety test after mild heat†	Mouse potency types I	Mouse potency types II	Mouse potency types III	Monkey potency types I	Monkey potency types II	Monkey potency types III
11	7.9	400	20	5		0/1	16	64	8	44	93	69
		"	20–20	5		0/3	4	64	4	4	102	8
		"	"	10			4	32	4	4	74	4
Control‡							4	4	16			
12	7.9	600	20	10		0/2	16	64	32	18	525	212
		"	20–20	5		0/5	32	32	64	19	131	11
Control§							4	4	32	65	290	130
" ‖							64	32	256			
14	6.1	800	20	20		0/5	8	32	32			
		"	20–20	5		"	8	32	32	8	35	1
		"	"		3	0/2	8	16	16			
Control¶							4	8	16	12	64	4
16¶	6.0	600	20		10	0/5	64	128	128			
		"	10–10		10	"	64	128	64			
Control¶							8	64	128			
17¶	6.2	600	20		10	0/5	64	128	64			
		"	20–20		3	"						
		"	"		5	"						
		"	"		8	"	16	256	64			
Control¶							16	64	128			
18¶	7.1	600	20–10		7	0/45	16	16	32			
Control¶							16	64	32			

* Each individual number signifies incident energy of irradiation; single number indicates one irradiation; double number, 2 irradiations; see footnote * Table III.

† In tissue culture bottles, 50 ml per bottle: Bottles negative cytopathogenically/bottles inoculated.

‡ Undiluted formalinized vaccine, supplied by Parke, Davis and Co., unless otherwise indicated, used in all experiments.

§ Control vaccine (‡) diluted 1:4.

‖ Live virus suspension diluted 1:5.

¶ Both test vaccine and control vaccine (‡) diluted 1:4.

combination of ultraviolet irradiation and another physical method, incubation, was studied in these experiments. The results indicate that exposure of virus suspensions to ultraviolet energy sufficient to reduce the initial titer 4 to 6 logs, followed by incubation, appears to produce satisfactory vaccines.

The reasons for the effectiveness of the ultraviolet-mild heat combination in producing a non-cytopathogenic suspension are not entirely clear. It may be that secondary inactivation is responsible and that this proceeds more rapidly at temperatures of 37° to 45°C. An alternative hypothesis is that virus particles which are altered(8) or injured by the ultraviolet, but not completely killed, more rapidly become non-cytopathogenic at the incubator temperatures. Of course, other explanations are possible, and

TABLE V. Stability of Vaccines Stored at 4°C. Antibody titers (mouse).

Vaccine No.	Months of storage	Dilution of vaccine	Virus type I	Virus type II	Virus type III
TV7	0	Undil.	32	32	64
		1: 5	16	4	16
		1:25	0	4	8
	10	Undil.	8	32	16
		1: 5	4	4	16
		1:25	0	0	0
8	0	Undil.	8	32	32
		1: 5	8	16	8
		1:25	8	16	4
	7	Undil.	16	32	32
		1: 5	4	16	4
		1:25	0	0	0
9	0	Undil.	8	32	16
		1: 5	4	4	4
		1:25	4	4	4
	6	Undil.	16	32	32
		1: 5	0	8	16
		1:25	0	0	0

subsequent work in progress is designed to explore them.

Summary. 1. Poliomyelitis virus of high titer in the form of a freshly filtered tissue culture preparation, can be inactivated by ultraviolet irradiation in centrifugal filmers. 2. Inactivation of the virus preparations by a combination of ultraviolet irradiation and exposure to mild heat for several days yields vaccine of good potency. In this procedure there is a margin of safety between complete inactivation and appreciable loss of antigenicity. 3. Experience thus far indicates a degree of consistency and reproducibility for the method described. 4. Vaccines made by a combination of ultraviolet irradiation and mild heat appear to retain their antigenicity for at least 7 months.

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Bruised Tissue. I. Biochemical Changes Resulting from Blunt Injury.* (23185)

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A bruise is a tissue injury without laceration usually produced by a blunt object resulting in rupture of the vascular supply and accumulation of blood and fluid. The accumulated blood undergoes, during the healing process, various physical and biochemical changes, represented by the color alteration characteristic of a bruise. Williamson(1) stated that the biochemical studies on wounds and wound healing have been devoted to the metabolism by the injured organism and the determination of the character and metabolism of certain proteins and polysaccharides of the wound area. In this report studies were undertaken to investigate the nature of some of the physical and biochemical changes occurring during the healing process of a bruised tissue.

Materials and methods. Bruising and sampling. Fifty-five cattle were used. The areas to be bruised and symmetrically located control areas were clipped and shaved. Injury was inflicted by 2 blows of a 7 pound sledge hammer over 3 feet in one-half second. (The degree of injury inflicted experimentally was less than that often sustained by cattle due to falls or contacts with other animals and sides of vehicles while in transit.) Observations were made of the bruised area for 9 days after injury. At various intervals animals were slaughtered and the bruised and control areas were excised, sliced, minced, and subjected to various chemical and physical studies. *Easily split iron* (E.S.I.). The tissue sample was homogenized with 0.4% HCl and incubated 24 hours at 37°C(2) and the E.S.I. was determined as the ferrous compound of o-phenanthroline(3). *Pigment extraction.* Saline, buffered to pH 7.4 and con-

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