FURTHER STUDIES ON EFFECTS OF TISSUE EXTRACTS ON STAPHYLOCOCCUS AUREUS

By LEO G. NUTINI, M.D., and SISTER EVA MARIA LYNCH

(From the Laboratories of the Institutum Divi Thomae, Cincinnati)

(Received for publication, March 13, 1946)

Recent research has stressed the toxigenic potentiality of the various strains of *Staphylococcus aureus*, demonstrating the manifold effects of *Staphylococcus aureus* in producing dermonecrosis, hemolysis, and death (1).

It is the general opinion among those who have surveyed the peculiar trends of bacteria that chromogenesis and other characteristic activities of these one-celled organisms will function only when environment is most favorable. This has been demonstrated with *Staphylococcus aureus* in the conversion of the virulent normal S yellow colony to the avirulent white R configuration by providing unfavorable conditions for growth with the simple addition of certain dyes and salts to the media (2). This modification was temporary, and passage *in vivo* inevitably terminated with the reversion of the variant form to the original type of colonial growth (2, 3).

Recently, work in these laboratories has shown that when brain (4) or spleen (5) extract is incorporated in the media, the yellow S is converted to the white R form and is so maintained in repeated subcultures on this same media. Moreover, conversion *in vivo* has been produced by injection of brain extract into animals infected with the yellow S organism (4). It was these findings which led us to conduct the present more extensive investigations of brain and spleen extracts, as well as of heart and kidney extract. The studies included (*a*) determination of the pathogenicity of the variant of *Staphylococcus aureus;* (*b*) the prevention and treatment of staphylococcic infections with the various extracts, using different methods of inducing infection and of administering the extracts; (*c*) a study of the mechanism of the action of the extracts both on toxin production by the organism, and on the toxin; and (*d*) determination of the acute and chronic toxicities of the extracts.

EXPERIMENTAL

Comparison of Pathogenicity of S and R Forms of Staphylococcus aureus. In vitro Studies.—Extraction and alcohol-precipitation of heart and kidney tissues from beef were made according to our original procedure (6) for spleen and brain except that distilled water was substituted for Drew's solution. All extracts, prior to further use, were checked for conversion activity *in vitro* on a virulent strain of *Staphylococcus aureus* cultured from an infected tonsil. The results

with all of the extracts were typical of those in previous experiments (5). In a series of three experiments the various tissue extracts were added in concentrations of 0.5 and 1.0 per cent to the solid nutrient media. As compared with the control plates, there was a depression in the growth rate of the inoculant, and a regression in the size of its original area. In the experimental plates this was followed at the end of 3 days by markedly increased growth, emanating from the original area of inoculation, of a white variant which within 2 days was approximately 3.5 to 5 times greater in area than that in the control plates. The effects were most marked with brain extract.

A series of tests (7-11) designed to distinguish between pathogenic and non-pathogenic strains of *Staphylococcus aureus*, were conducted in triplicate on both the S and R forms. From the data in Table I it would appear that the white R variant is non-pathogenic. Tests for chromogenesis made at intervals during $2\frac{1}{2}$ years show that the variant has never reverted to the original orange growth.

TABLE I

Tests for Distinguishing between Virulent and Avirulent Forms of Staphylococcus aureus

Tests	Control—yellow organism	Experimental—gray-white organism
Fermentation of mannitol	+	−
Liquefaction of gelatin	+	+ very slight
Chromogenesis	+ yellow	− gray white
Hemolysis	+	−
Coagulase	+	−

Although none of the applied "*in vitro*" tests serves as a standard whereby a line of demarcation between virulence and avirulence can be drawn, some investigators maintain that negative reaction to a combination of these tests is a fairly accurate indication of non-pathogenicity (1,3, 7-14).

In vivo Studies.—To determine whether the avirulence of the white R variant could be maintained *in vivo*, mice, 3 to 6 months old, BBC strain, and albino, were used as test animals. From the 3 to 5 day old experimental plates, yellow organisms were obtained from the center of the inoculation area and white organisms from the stimulated outgrowth from this area. Each test animal was immediately injected subcutaneously with 1 ml. of saline containing 1 loopful of the organisms. The experiments were made in triplicate using 10 animals for the yellow and 10 for the white organisms in each experiment. Three days after injection of the yellow organism, the control animals developed a gradually ascending paralysis, with 70 per cent mortality by the 4th day. The remaining 30 per cent developed large abdominal abscesses and sloughs which persisted for 3 weeks. There was no mortality among the experimental animals receiving the white organisms. These animals did not develop paralysis, and only 20

per cent had small non-suppurative hemorrhagic abdominal lesions which disappeared by the 7th day. Cultures from the lesions in the survivors in each of the two groups of animals revealed organisms corresponding in type to those injected, evidence for the maintenance of the relative avirulence *in vivo* of the white R variant of the strain of *Staphylococcus aureus* used in these experiments.

Treatment of Staphylococcic Infections with Tissue Extracts

Procedure. Control Animals.—In the following experiments extending over a period of 18 months, BBC and albino mice of the Rockland strain 3 to 6 months of age were used. The strain of *Staphylococcus aureus* employed was one obtained from the drainage of an infection on the hand and the organisms were maintained in nutrient broth as culture medium. At the time of use for infecting mice, saline suspensions were prepared with 1 loopful of a 48 hour culture of the organism per cc. of saline. The LD_{50} was 0.35 cc. Lesions developed within 1 to 3 days following subcutaneous injection into the ventral abdominal region and in the surviving animals required 9 to 30 days to heal. Abscess formation was followed by extensive sloughing and the appearance of signs of intoxication, listlessness, inability to eat, shaggy hair, cyanosis, paralysis of the limbs, and death within 5 to 6 days. Blood cultures made from animals dying then showed that death was due to the staphylococcus. Following intraperitoneal (0.25 and 0.5 cc.) or intravenous (0.1 cc.) injection of the organisms there were no localized lesions, but a generalized reaction occurred characterized by roughened fur, listlessness, sluggishness, and reduced activity. Death occurred in these animals within 24 hours and in some instances within a few minutes following the injection. The survivors required 14 and 15 days for recovery.

Experimental Animals: Brain Extract.—Brain extract was given subcutaneously in the ventral abdominal region each day to mice for (1) prophylactic experiments 2 to 6 hours prior to infection with *Staphylococcus aureus* and (2) for therapeutic experiments on the 1st day that lesions developed following injection, usually after 1 to 3 days, or, in the case of intravenous and intraperitoneal infection, following the onset of the generalized reaction. Treatment was given daily in 50 mg. doses until the lesions healed completely, as judged by scabs dropping off leaving smooth new skin beneath, or until the animals appeared normal following a generalized reaction. In the therapeutic experiments the animals were divided into control and experimental groups only after the lesions or the generalized reaction occurred, in order to have animals with infections of similar degree of severity in each group. The oral effectiveness of brain extract was tested both therapeutically and prophylactically using 300 mg. per 24 hours administered at intervals of 6 hours, through a curved hypodermic needle with a blunt edge.

In another series of experiments the relative effectiveness of decreasing amounts of the brain extract was tested against staphylococcic infections grading from a 50 mg. dose of the brain extract down to 5 mg. daily in both a prophylactic and therapeutic series of experiments.

The control animals were injected each day with a volume of saline equivalent to that of the brain extract.

Results.—In 25 experiments (Table II) using 50 mg. of brain extract daily in the prophylactic treatment of subcutaneous staphylococcic infections (227 control and 223 experimental mice) all of the 25 per cent of the controls surviving the infection developed typical abscesses which required from 9 to 30 days to heal. The incidence of lesions in the 99 per cent surviving experimental animals was 63 per cent. The interval between injection of the organisms and the

TABLE II

Effect of Brain Extract Administered Subcutaneously as Prophylaxis against Subcutaneous Staphylococcus aureus Infections in Mice

Experiment No.	No. mice	Mortality	Lesions—survivors		
			Average day appeared	Frequency	Healing time Average (range)
		per cent		*per cent*	*days*
5314 BP	10C	60	3	100	12 (2 unhealed 14th day)
	10E	0	8	60	4 (3–6)
6194 BP	9C	44	6	100	17–25
	9E	0	9	55	7 (6–8)
7134 BP	10C	70	5	100	10
	10E	0	5	20	3
3265 BP	10C	100	—	—	—
	10E	0	4	50	9 (4–11)
545 BP	10C	80	3	100	22
	10E	0	2	20	6 (5–7)
1105 BP	6C	33	2	100	16 (15–20)
	6E	0	2	33	8 (7–9)
355 BP	6C	33	3	100	28 (27–30)
	6E	0	4	50	8 (5–11)
3125 BP	6C	100	—	—	—
	6E	0	3	100	7 (6–11)
545 BP	10C	70	3	100	21 (18–23)
	6E	0	3	100	8 (5–10)
6255 BP	6C	83	2	100	20
	6E	0	2	67	9 (7–13)
625 BP	6C	83	2	100	23
	6E	0	2	67	5 (3–6)
1105 BP	6C	33	2	100	18
	6E	0	2	67	7 (5–9)
2195 BP	10C	60	3	100	17 (10–25)
	10E	0	4	50	6 (4–9)
5255 BP	10C	70	3	100	17 (12–21)
	10E	0	3	60	8 (4–10)

TABLE II—*Concluded*

Experiment No.	No. mice	Mortality	Lesions—survivors		
			Average day appeared	Frequency	Healing time Average (range)
		per cent		*per cent*	*days*
1025 BP	10C	100	—	—	—
	10E	0	2	50	8 (6–10)
9115 BP	10C	70	2	100	15 (9–20)
	10E	0	2	60	5 (3–8)
10115 BP	10C	90	2	100	22
	10E	0	2	50	5 (4–8)
10245 BP	6C	100	—	—	—
	6E*	0	2	83	5 (4–6)
11145 BP	10C	100	—	—	—
	10E	0	2	80	6 (4–8)
11145 BP	10C	90	2	100	25
	10E	0	3	50	6 (3–10)
11305 BP	10C	90	3	100	Unhealed on 20th day
	10E	0	3	90	7 (3–11)
12105 BP	10C	80	2	100	20 (1 unhealed on 21st day)
	10E	0	2	60	7 (4–10)
12105 BP	6C	67	2	100	9 (1 unhealed on 11th day)
	6E	0	2	60	8 (6–10)
5255 BP	10C	40	3	100	19 (16–22)
	10E	0	3	70	12 (8–14)
6255 BP	20C	95	3	100	17
	20E	20	7	100	6 (3–8)
Totals and	227C	75.0	3	100	18 (9–30)
averages	223E	0.9	3	63	7 (3–14)

Prophylactic dose of 50 mg. brain extract subcutaneously given 2 to 6 hours prior to inoculating with virulent *Staphylococcus aureus*, 1.5 LD_{50} subcutaneously. Treatment continued at 50 mg. level daily until lesions completely healed, as gauged by the loosening of the scab beneath which was a shining new intact skin surface.

* Animals ill. No open lesions; local inflammatory reaction; regression in 5 days.

appearance of the abscesses was not significantly altered in the experimental animals. The abscesses, however, were atypical in appearance, consisting of small dry lesions which did not progress to the sloughing stage and which healed completely in time intervals ranging from 3 to 14 days. There was no paralysis or manifestation of toxic symptoms in the treated animals.

In 11 therapeutic experiments using a total of 116 control and 116 experimental animals (Table III), in which treatment was begun with 50 mg. of brain extract daily after the typical lesion following subcutaneous injection of *Staphylococcus aureus* had developed, 87 per cent of the control animals and 3 per cent of the experimental animals died. The range of healing time for the surviving control animals was 14 to 26 days with typical toxic manifestations and for the experimental animals, 4 to 15 days, in the absence of symptoms of toxicity.

The results of treating intraperitoneal infections in 50 mice by subcutaneous injection of 50 mg. of brain extract per day, both prophylactically and therapeutically, are shown in Table IV. In the prophylactic series, two-thirds of the 30 control animals died and the survivors required 14 to 19 days to recover from the generalized reaction. Of the 30 experimental animals that received a single preliminary dose of 50 mg. of brain extract subcutaneously, followed by 50 mg. per day, one died (1185 BP; the other 9 animals in this group did not react to the injection of the organisms in any apparent way) and the remaining experimental animals seemed normal again within 3 to 8 days. In the therapeutic series, none of the 20 control animals survived the intraperitoneal injection of organisms; none of the experimental animals died and they appeared to be completely recovered within 3 to 5 days.

None of 35 experimental animals died that received *Staphylococcus aureus* intravenously (Table V) and brain extract subcutaneously whether prophylactically or therapeutically. The single survivor of the 35 control animals required 15 days for recovery from the generalized reaction. Three, 4, and 6 days were required for recovery of the experimental animals with the exception of one group prophylactically treated (10205 BP) in which there was an immediate generalized reaction of all of the animals to the intravenous injection, but within 15 to 30 minutes all had apparently recovered. Treatment was continued for 3 days in this group. All of the control animals died within 24 hours of inoculation.

In Table VI are given the results of using doses of brain extracts graded to as low as 5 mg. per day prophylactically in a series of 6 groups of 6 experimental animals each, and 1 group of 6 control animals. There was no mortality among the experimental animals until the amount of brain extract was reduced to 10 and 5 mg. per day, while the healing time was gradually lengthened with the daily administration of smaller amounts. The single surviving control animal required 23 days for the healing of the lesion. In the therapeutic experiments of

TABLE III

Therapeutic Response of Mice with Subcutaneous Staphylococcus aureus Infection to Subcutaneous Treatment with Brain Extract

Experiment No.	No. mice	Mortality	Survivors Healing time Average (range)
		per cent	*days*
10194 BT	10C	100*	—
	10E	0	8 (7–13)
625 BT	10C	100	—
	10E	0	8 (6–9)
1295 BT	6C	83	23
	6E	0	9 (7–12)
755 BT	10C	50	16 (10–20)‡
	10E	0	6 (4–9)
9115 BT	10C	80	18 (14–22)
	10E	0	8 (4–12)
9295 BT	10C	100	—
	10E	0	7 (5–10)
10115 BT	10C	100	—
	10E	0	8 (4–11)
11145 BT	10C	100	—
	10E	10	7 (4–9)
11165 BT	10C	70	18 (15–21)
	10E	0	9 (6–13)
525 BT	10C	80	25 (23–26)
	10E	0	10 (4–15)
6255 BT	20C	95	14
	20E	15	9 (6–13)
Totals and.....	116C	87	19 (14–26)
averages.....	116E	3	8 (4–15)

Subcutaneous dose of *Staphylococcus aureus* equivalent to approximately 1.5 LD_{50}.

Treatment begun only when all animals had open lesions, usually on 2nd or 3rd day after injection with 1.5 LD_{50} of *Staphylococcus aureus* and continued until complete healing occurred, using 50 mg. of brain extract subcutaneously once daily.

* Mortality 80 per cent at 21 days, 100 per cent at end of 6 weeks, due to secondary outbreaks of infection.

‡ One unhealed on 22nd day.

the same series, there were no deaths in the 6 groups of 10 experimental animals each, while all of the 10 control animals died. The healing time with this, as with the prophylactic series, was gradually lengthened as the daily amount of brain extract injected was reduced.

TABLE IV

Response of Mice to Subcutaneous Injections of Brain Extract as Prophylactic and Therapeutic Treatment of Intraperitoneal Infections with Staphylococcus aureus

Experiment No.	No. Mice	Mortality	Survivors—illness		
			Day of onset	Frequency	Recovery time Average (range)
Prophylaxis					
		per cent		*per cent*	*days*	*days*
1185 BP	10C	100	—	—	—	—
	10E	10	No illness manifested			
2185 BP	10C	40	3	100	14	(14)
	10E	0	3	100	3	(3)
11244 BP*	10C	80	1	100	23	(23)
	10E	0	1	100	8‡	(8)
Totals and.....	30C	67	2	100	19	(19)
averages.....	30E	3	2	100	6	(6)
Therapeusis						
11154 BT§	10C	100	—	—	—	—
	10E	0	1	100	3	(3)
11154 BT‖	10C	100	—	—	—	—
	10E	0	1	100	5	(5)
Totals and.....	20C	100	—	—	—	—
averages.....	20E	0	1	100	4	(4)

* All surviving control animals developed abscesses at site of injection; 3 experimental animals had slight sloughs at site of injection which healed within 11 days.

§ 0.25 cc. inoculant used. Experimental animals treated for 10 days.

‖ 0.5 cc. inoculant used.

‡ Dry slough appeared at site of injection on day 9 in 3 animals; healed 11 days later.

The oral effectiveness of brain extract therapy for subcutaneous *Staphylococcus aureus* infections is demonstrated by the data in Table VII. None of the treated animals in either the prophylactic or the therapeutic series died. All of the controls for the therapeutic series died. The two surviving controls in the prophylactic series required 15 and 21 days for healing of the abscesses.

In the 10 experimental animals receiving a single prophylactic dose of 100 mg. of brain extract *per os* only one developed an abscess, which appeared 5 days after subcutaneous infection with *Staphylococcus aureus*; lesions developed in all of the control animals of this series on the 2nd day after infection.

Other Tissue Extracts.—Therapeutic tests on subcutaneous infections with the other beef tissue extracts—liver, spleen, heart, and kidney—were accompanied by results less striking than those with brain extract (Table VIII;

TABLE V

Response of Mice to Subcutaneous Injections of Brain Extract as Prophylactic and Therapeutic Treatment of Intravenous Infection with Staphylococcus aureus

Experiment No.	No. mice	Mortaliy	Survivors—illness		
			Day of onset	Frequency	Recovery time Average (range)
Prophylaxis					
		per cent			*days*
195 BP	10C	100	—	—	—
	10E	0	1	100	3 (2–3)
10205 BP	9C*	100	—	—	—
	9E‡	0	Immediate reaction of all animals with improvement in 15–30 minutes.		
Therapeusis					
195 BT	10C	100	—	—	—
	10E	0	Immediate	100	6 (4–8)
213 BT	6C	83	1	100	15 (15)
	6E	0	1	100	4 (3–4)

Animals inoculated with 0.1 cc. of a 24 hour culture of organism.

* All dead within 24 hours of inoculations.

‡ Treatment continued for 3 days.

Fig. 1). There was no mortality when the spleen and the heart extracts were used, but a slightly longer time (7 to 12 and 5 to 16 days, respectively) was required for complete healing of the lesions than was the case with brain extract (3 to 15 days). The infection in animals treated with kidney extract, however, showed but slight improvement as compared with that of the controls and it was felt that the poor results in this case may have been due in some measure to the toxicity of the extract at the dosage used, since all animals treated with the kidney extract exhibited toxic manifestations immediately following injections of the material. The liver extract proved too toxic to warrant further investigation.

TABLE VI

Effect of Various Subcutaneous Doses of Brain Extract on Subcutaneous Straphylococcus aureus Infections in Mice

Dosage, *mg./day*	50*	40	30	20	10	5	Controls
Prophylactic							
No. mice	6	6	6	6	6	6	6
Mortality, *per cent*	0	0	0	0	16	33	83
Survivors							
Average day for lesions	2	2	2	2	2	2	2
Frequency, survivors, *per cent*	67	67	83	100	100	100	100
Healing time, average, *days*	5	5	8	6	9	10	23
Range, *days*	(3–6)	(4–8)	(4–11)	(4–10)	(5–15)	(8–13)	—
Therapeutic‡							
No. mice	10	10	10	10	10	10	10
Mortality, *per cent*	0	0	0	0	0	0	100
Survivors							
Healing time, average, *days*	7	7	11	9	11	11	—
Range, *days*	(5–10)	(4–10)	(3–17)	(5–13)	(8–13)	(7–13)	

* Data included in both Tables II and III.

‡ Abscesses in 100 per cent control and experimental animals on 4th day following subcutaneous inoculation of *Staphylococcus aureus*. Brain extract therapy was begun daily on day 4 and continued until complete healing occurred as defined in Table II. Dose of inoculant 1.5 LD_{50}.

TABLE VII

Response of Mice with Subcutaneous Staphylococcus aureus Infections to 300 mg. per day of Brain Extract Administered Orally

Experiment No.	No. mice	Mortality	Lesions—survivors		
			Average day appeared	Frequency	Healing time Average (range)
Prophylaxis					
		per cent		*per cent*	*days*
2195 BP	10C	80	2	100	18 (15–21)
	10E	0	5	10	5 (5)
Therapeusis					
3265 BT	10C	100	—	—	— —
	10E	0	1	100	8 (4–11)
Totals and...	20C	90	2	100	18 (15–21)
averages....	20E	0	3	55	7 (4–11)

Brain extract administered at intervals of 4 hours through a curved needle directly into the stomach, a total of 300 mg. per day being given. *Staphylococcus aureus* dose was 1.5 LD_{50}.

TABLE VIII

Response of Mice to Prophylactic and Therapeutic Subcutaneous Administration of Liver, Heart, Kidney, and Spleen Extracts for Control of Staphylococcus aureus Infections in Mice

	Liver		Heart		Kidney		Spleen	
	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
Prophylaxis								
No. mice	6	6	10	10	10	10	6	6
Mortality, *per cent*	67	83	50	60	60	10	33	0
Survivors-lesions								
Average day of appearance	2	2	1	2	1	2	2	2
Frequency, *per cent*	100	100	100	100	100	55	100	67
Healing time, average	16	22	15	13	15	9	18	8
Range	(16)	(22)	(14–15)	(12–16)	(14–17)	(8–9)	(18)	(7–8)
No. mice			6	6	6	6	6	6
Mortality			33	0	33	17	83	0
Survivors-lesions								
Average day of appearance			2	2	2	2	2	2
Frequency, *per cent*			100	83	100	83	100	100
Healing time, average			18	8	18	11	23	10
Range			(18)	(5–13)	(18)	(7–15)	(23)	(10)
Totals and averages for prophylactic experiments — No. mice	6	6	16	16	16	16	12	12
Mortality	33	83	45	40	50	13	44	0
Healing time	4	—	18	10	17	10	21	9
Range	(4)		(18)	(5–11)	(14–18)	(8–15)	(18–23)	(7–10)
Therapeusis								
No. mice	6	6	6	6	6	6	6	6
Mortality	83	83	83	0	83	83	83	0
Survivors-lesions								
Average day of appearance	2	2	3	3	1	1	1	1
Frequency, *per cent*	100	100	100	100	100	100	100	100
Healing time, average	23	25	23	10	23	11	23	11
Range	(23)	(25)	(23)	(7–14)	(23)	(11)	(23)	(11–12)

Subcutaneous infections induced with 1.5 LD_{50} *Staphylococcus aureus.*

Mechanism of Action of Tissue Extracts on Staphylococcus aureus in Vivo

The following investigations are concerned with the effects of the tissue extracts on toxin production by *Staphylococcus aureus* and on the action of the toxin.

Extracts and Toxin Production.—Control tubes containing nutrient broth and experimental tubes containing nutrient broth plus 1.0 per cent brain extract were inoculated with *Staphylococcus aureus* and allowed to incubate for 48 hours. Both sets of tubes were then centrifuged slowly for 15 minutes at the end of which time the supernatant materials were subjected to several filtrations through Berkefeld filters.

To determine whether brain extract prevented the formation of toxin by *Staphylococcus aureus* (15), the experimental and control filtrates were injected into test mice. The control filtrate was divided into 2 parts and 1.0 per cent brain extract was added to one portion to eliminate any variable which might have been introduced by the presence of brain extract in the experimental filtrate. In two series of experiments 3 groups of 10 mice each were injected subcutaneously in the abdominal region with 0.5 ml. of each of the filtrates. Observations on necrosis, hemolysis, and death are recorded in Table IX. The control filtrate was markedly toxic both as to its power to necrose tissue and to produce hemolysis, while that

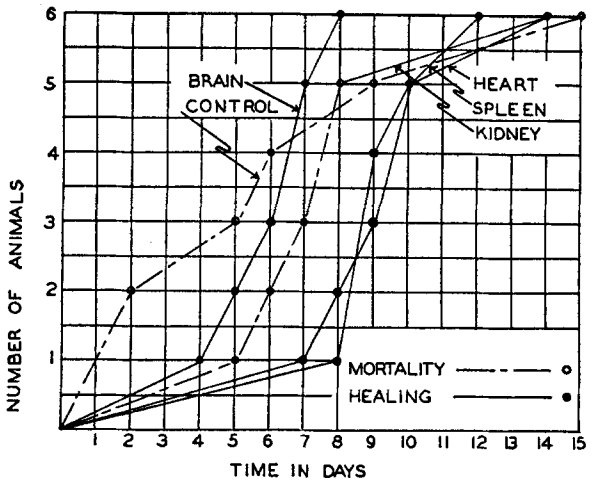

FIG. 1. Comparison of therapeutic action of brain, spleen, heart, and kidney extracts on staphylococcic infections initiated subcutaneously.

prepared from organisms grown under the influence of the brain extract was non-toxic. It is apparent from the data presented that the hemolytic and necrotizing effects of toxin, while not eliminated, were considerably reduced by the simple addition of brain extract to the control filtrate.

To check for the lethal action of the toxin of *Staphylococcus aureus*, 3 groups of 10 mice each were inoculated intravenously with the three filtrates, each mouse receiving 0.5 ml. There was almost immediate total mortality in the animals receiving the control filtrate and those receiving the control filtrate to which brain extract had been added, but there were no ill effects in those animals injected with the filtrate from organisms grown in the presence of brain extract.

The cholesterol content of the extract was 0.032 mg. per dose and the phospholipid content was too small to determine. The content of these substances is too small to account for the action of the extract against the strain of *Staphylococcus aureus*. Preliminary experiments indicate that the protection of the

extracts against the infection is not due to a non-specific effect such as leukocytosis. Apparently, therefore, the effectiveness of brain extract in combatting *Staphylococcus aureus* infections in animals is due in great measure to the pre-

TABLE IX

Effect of Beef Brain Extract on the Toxin Production by Staphylococcus aureus as Tested in Mice

Type of filtrate	Mortality	Average hemoglobin 4th day of infection	Average R.B.C. 4th day of infection	Necrosis
	per cent	*per cent*		
Toxin filtrate of *Staphylococcus aureus*, control	100	23.4	2,136,000	10, severe
Toxin filtrate of *Staphylococcus aureus*, control plus extract	60	54.0	4,448,000	5, moderate 5, slight
Toxin filtrate of *Staphylococcus aureus*, experimental	10	91.2	9,625,000	2, slight 8, none

Ten mice in each series, weight of animals 20 gm.

TABLE X

Acute Toxicity of Beef Tissue Extracts in Mice over a 10 Day Period

Type of extract	No. mice*	Mortality at different dosages‡						Average weight gain or loss					
		50 mg.	100 mg.	200 mg.	300 mg.	400 mg.	500 mg.	50 mg.	100 mg.	200 mg.	300 mg.	400 mg.	500 mg.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Brain	12	0	0	0	0	0	100	+1.3	+1.15	+1.6	+0.8	+1.25	—
Spleen	12	0	0	0	0	0	100	−0.8	−0.2	+0.9	−1.1	−1.6	—
		20 mg.	40 mg.	80 mg.	140 mg.	200 mg.		20 mg.	40 mg.	80 mg.	140 mg.	200 mg.	
Heart	10	0	0	50	50	50		−0.1	+0.15	−1.2	−1.0	−3.2	
Kidney	10	0	0	50	50	0		−0.9	−1.7	−1.9	−0.9	−6.3	

* Average weight, 20 gm.

‡ Daily injections, subcutaneously.

vention of toxin formation and only slightly to action on the formed toxin. Experimental study of the rôle of the tissue extracts in the general immunologic picture is in progress.

Toxicity of Extracts

Since previous experiments indicated that extracts of brain and spleen were less toxic than those of heart and kidney, slightly higher dosage levels of the

former were used in acute toxicity tests. The results demonstrate the greater toxicity of the heart and kidney extract (Table X). Doses smaller than 500 mg. of brain and spleen extract, a dosage approximately equivalent to 2 to 2.5 per cent of the body weight, did not kill the animal. Injections of the spleen extract were accompanied by evidence of toxicity in the form of weight loss. Heart and kidney extracts, in addition to producing marked weight loss, resulted in mortality with dosages as low as the 80 mg. level, an amount approximately only 0.3 per cent of the body weight.

The tests for chronic toxicity (Table XI), likewise showed brain and spleen extracts to be the safer materials. The low mortality in animals receiving brain

TABLE XI

Chronic Toxicity of Beef Tissue Extracts in Mice over a 60-Day Period

Type of extract	No. Mice*	Extract dosage‡	Average weight gain	Experimental interval before death	Mortality
		mg.	*gm.*	*day*	
Brain	10	50	5.5	50th–58th	2
	10	100	7.1	58th	1
Spleen	10	50	5.9	27th–39th	5
	10	100	4.3	25th–55th	6
Heart	6	20	—	35th–42nd	4
	6	40	—	26th–41st	6
Kidney	6	20	—	27th–40th	6
	6	40	—	8th–30th	6

* Average weight, 20 gm.

‡ Daily injections, subcutaneously

extract occurred only within the last few days of the 60 day experiment. Mortality from the use of spleen and heart extracts was greater than with brain, and occurrred in the third quarter of the experimental interval. Mortality with the spleen extract was somewhat lower than that for the heart extract even though it was given at a higher dosage level. The kidney extract produced 100 per cent mortality with both dosages within 40 days after beginning injections.

SUMMARY

1. The ability of alcoholic-precipitated extracts of beef tissue—brain, spleen, heart, and kidney—to stimulate the growth of *Staphylococcus aureus, in vitro*, and to convert the yellow S form to a white R variant with altered biochemical characteristics conforming to those of an avirulent organism, has been confirmed.

2. The avirulence of the white R variant has been established by tests *in vivo* on mice.

3. *Staphylococcus aureus* infections induced subcutaneously, intraperitoneally, and intravenously in mice responded favorably to brain extract following subcutaneous or oral administration. The mortality was 2 per cent in 444 experimental animals and 81 per cent in 448 control animals.

4. The extracts appeared equally efficient when used therapeutically (mortality 2 per cent of 162 experimental animals and 90 per cent in the control series) or prophylactically (mortality 2 per cent of 282 experimental animals and 76 per cent in 286 control mice). Extracts of brain and spleen were more effective than those of either heart or kidney.

5. Studies concerning the mechanism of action of the tissue extracts indicate that they prevented the formation of toxin by *Staphylococcus aureus*, and had but little effect on toxin actions.

6. Toxicity tests revealed that the brain and spleen extracts were relatively non-toxic, dosages equivalent to 2 per cent of the body weight being well tolerated. Kidney and heart extracts were much more toxic, producing mortality in dosages as low as 0.3 per cent of the body weight.

BIBLIOGRAPHY

1. Blair, J. E., *Bact. Rev.*, 1939, **3**, 97.
2. Hoffstadt, R., and Youmans, G. P., *J. Infect. Dis.*, 1932, **51**, 216.
3. Pinner, M., and Voldrich, M., *J. Infect. Dis.*, 1932, **50**, 185.
4. Nutini, L. G., and Lynch, E. M., *Nature*, 1945, **156**, 419.
5. Nutini, L. G., Kreke, C. W., and Schroeder, M. P., *J. Bact.*, 1945, **50**, 177.
6. Nutini, L. G., and Kreke, C. W., *J. Bact.*, 1942, **44**, 661.
7. Chapman, G. H., Berens, C., Curcio, L., and Nilson, E. L., *J. Bact.*, 1937, **33**, 646.
8. Chapman, G. H., Berens, C., Curcio, L., and Nilson, E. L., *J. Bact.*, 1938, **35**, 311.
9. Chapman, G. H., Berens, C., Peters, A., and Curcio, L., *J. Bact.*, 1934, **28**, 343.
10. Chapman, G. H., Lieb, C. W., Berens, C., and Curcio, L., *J. Bact.*, 1937, **33**, 533.
11. Cowan, S. T., *J. Path. and Bact.*, 1938, **46**, 31.
12. Cruickshank, R., *J. Path. and Bact.*, 1937, **45**, 295.
13. Dudgeon, L. S., and Simpson, J. W. H., *J. Hyg.*, Cambridge, Eng., 1928, **27**, 160.
14. Holman, W. L., *Am. J. Med. Sc.*, 1935, **189**, 436.
15. Burnet, F. M., *J. Path. and Bact.*, 1930, **33**, 1.