ents cardol, anacardic acid and a decarboxylated derivative of anacardic acid called anacardol. Cardol is a derivative of resorcinol with a 15-carbon unsaturated side chain (average of two double bonds) in the 5-position, whereas the other two compounds are derivatives of 2-carboxy phenol and phenol, respectively, with a similar unsaturated side-chain in the 3-position.[4] The monophenolic component isolated commercially is known as "cardanol."[5,6]

The problem of hypersensitiveness to cashew nut shell liquid and to its various fractions and derivatives has engaged our attention during the past year.[7] As a result of studies involving more than 150 patients, we have found that persons sensitive to poison ivy are also sensitive to cardol in practically all instances, and to the raw oil, anacardic acid, anacardol and "cardanol" in the majority of cases. Of the ingredients in cashew nut shell liquid cardol elicits the most intense group reactions by far. Contrariwise, all patients showing a negative patch test to poison ivy gave a negative response to the cashew nut shell liquid and its components.

The similar reaction to poison ivy and to the components of the cashew nut shell liquid indicates a group reactivity that is of considerable interest. Thus, cardol, anacardic acid and anacardol all bear the long unsaturated side chain in the position *meta* to a phenolic hydroxyl group, which is likewise true for the catechol configuration of the poison ivy ingredient. Hydrogenating the double bonds in cardol, as in forming tetrahydrocardol, has the effect of diminishing the incidence and intensity of group reactions. Likewise, hydrogenating anacardic acid and anacardol has the same effect in diminishing the incidence and intensity of group reactivity. On the other hand, resorcinol compounds bearing a moderately long saturated alkyl side-chain in a position *ortho* or *para* to a phenolic hydroxyl group, as in hexyl resorcinol, seem not to show this group reactivity. Likewise, when the side-chain is in a position *meta* to the phenolic hydroxyl group, we have found that the reactivity is dependent on the length of the side-chain. Thus, compounds bearing a short side-chain in the 3-position of catechol show only mild group reactivity, and in the 4-position no reactivity. This difference between the 3 and 4 position of catechol, even though both are *meta* to phenolic hydroxyl groups, should be stressed, since a number of biochemical products are catechol compounds with short-chain substituents in the 4-position, such as adrenalin and 3,4-dioxyphenylalanine.[7]

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[4] H. J. Backer and N. H. Haack, *Rec. Trav. Chim.*, 60: 661, 1941.

[5] M. T. Harvey and S. Caplan, *Ind. Eng. Chem.*, 32: 1306, 1940.

[6] D. Wasserman and C. R. Dawson, *Ind. Eng. Chem.*, 37: 396, 1945.

[7] After the manuscript for this article had been submitted to the editors of SCIENCE, there appeared in the June issue of *Industrial Medicine* (14: 500, 1945) an article by Dr. Louis Schwartz and collaborators dealing with the "Skin Hazards in the Manufacture and Use of Cashew Nut Shell Liquid—Formaldehyde Resins." Certain of the findings and conclusions made by Dr. Schwartz and his collaborators are not in agreement with those expressed in this article. Our criticisms of the article by Dr. Schwartz and collaborators have been submitted to the editor of *Industrial Medicine*.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

PRECIPITABLE TYPHOID SOMATIC ANTIGEN IN THE SERUM OF TYPHOID FEVER PATIENTS[1]

THE observations reported in this paper were made in 1940 and 1941 as part of a study of the mechanisms of pathogenesis of typhoid fever. The work was interrupted by more urgent war-time activities, and publication has been delayed. The selected data presented below are offered at this time because of its practical implications.

The presence of antigen of *Eberthella typhi* in the serum of a typhoid fever patient can readily be demonstrated near the onset of the disease by the simple procedure of layering a small quantity of the patient's serum on a like quantity of specific immune rabbit serum. In a positive test, a precipitate appears at the interface of the two sera.

MATERIAL

Immune rabbit serum: Specific antisera were available against the 0-907, Ty 2, Rawlins and Watson strains of *Eberthella typhi;* the somatic "O" agglutinin titers varied from 1:6,000 to 1:50,000, with flagellar "H" titers not exceeding 1:1,280. Although these high-titer sera were used for experimental purposes, it has been found that any specific immune rabbit serum, prepared by the injection of suspensions of *E. typhi,* is satisfactory if the "O" agglutinin titer is 1:2,560 or more. The serum should be col-

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TABLE 1

DEMONSTRATION OF PRECIPITABLE TYPHOID ANTIGEN IN PATIENT'S SERUM

Case No.	Day of illness	Serum precipitation	Agglutinin titers			Cultures*			Final clinical diagnosis
			Ty "O"	A–"O"	B–"O"	OX 19	Blood	Stool	
1 (35557)	3rd	++++	:40	0	:20	0	*Eb. typhi*	*Sh. paradysenteriae*	Typhoid fever and bacillary dysentery
	10th	+++	:160	:20	:20	–	0	*Eb. typhi*	
2 (35717)	5th	+++	:80	0	0	0	*Eb. typhi*	–	Typhoid fever
	20th	0	:640	:80	:40	–	0	*Eb. typhi*	
3 (PCL)	8th	+++	:160	0	0	:40	*Eb. typhi*	0	Typhoid fever
	14th	0	:640	0	:20	:80	0	*Eb. typhi*	
4 (35922)	9th	+++	:160	0	:20	:40	*Eb. typhi*	0	Typhoid fever
	18th	0	:640	0	:20	:80	0	*Eb. typhi*	
5 (35502)	20th	++	:80	:20	0	0	0 (3 trials)	0 (3 trials)	Typhoid fever with thrombophlebitis
6 (14354)	8th	++	:320	:80	:80	:40	*Eb. typhi*	0	Typhoid fever previous TAB vaccine
	32nd	0	:1280	:160	:640	:80	–	0	
7 (35945)	9th	++	:320	:80	:20	–	*Eb. typhi*	0	Typhoid fever previous TAB vaccine
	16th	0	:640	:80	:160	–	0	*Eb. typhi*	
8 (35997)	30th	++	:80	:20	0	:160	*Eb. typhi*	0	Typhoid† relapse
9 c (35607)	8th	0	"H" :320	"H" :20	"H" :1280	–	–	–	Paratyphoid B not vaccinated
10 c (35931)	10th	0	0	0	0	0	*Br. melitensis*	0	Brucellosis. Had had TAB annually
11 c (18429)	8th	0	:40	0	0	:20	0	0	Bronchitis
12 c (35556)	8th	0	:640	:160	:20	:640	0	0	Murine typhus recent TAB vaccine
13 c (35880)	8th	0	:80	:40	:20	:1280	0	0	Murine typhus no TAB vaccine

* 0 indicates no growth of significant organism in culture.
– indicates no specimen cultured.
† The blood yielded a positive culture on the 41st day; earlier and later blood cultures were negative, and cultures of the urine and stools were negative throughout.

lected aseptically, filtered through a Seitz filter and stored without preservative. The serum should be cleared of any precipitate by centrifuging it immediately before use.

Patient's serum is collected in the usual manner and cleared of all particles by centrifuging immediately before use. A quantitative receptor analysis should be made on the sample of patient's serum used for the precipitation test.

PROCEDURE

In order to conserve specific immune serum, use small tubes (3 × 30 mm) made from glass tubing. These tubes must be perfectly clean. By means of a capillary pipette, place about 0.1 cc of immune rabbit serum in the bottom of the tube, and by means of a second capillary pipette a similar quantity of patient's serum is carefully layered on top of the immune serum. Mixtures of the two sera usually fail to yield a precipitate, probably because of excess of antibody. Control tubes containing (1) immune rabbit serum plus normal human serum and (2) normal rabbit serum plus patient's serum should also be set up.

THE REACTION

In a positive test, a sharply defined plaque of precipitate is apparent at the interface of the two sera when the tube is held in a beam of strong light; in most instances the reaction appears almost immediately at room temperature, but in doubtful cases the tubes are incubated at 37° C. for one hour before a final reading is made. The amount of precipitation is recorded as 4 plus, 3 plus, 2 plus, 1 plus or negative. In a 4-plus reaction, a heavy flocculent precipitate appears immediately and rapidly settles to the bottom of the tube; lesser degrees of precipitation are given the relative values indicated. The presence of preservative may lead to false positive reactions.

RESULTS

Some degree of precipitation has been obtained with the sera of 32 bacteriologically proved cases of typhoid fever, and in 8 other cases where the clinical and serological diagnoses favored enteric fever, but where cultures of blood, feces and urine failed to yield growth of significant organisms. Representative febrile control cases tested between the fifth and tenth days of fever have been negative. The "control" group of 22 cases included proved infections with *Salmonella schottmuelleri* (paratyphoid B, 1 case), *Brucella melitensis* (Malta fever, 3 cases) and *Rickettsia prowazeki*, var. *mooseri* (murine typhus, 3 cases), as well as cases of bronchopneumonia, infectious hepatitis and non-typhoidal pyrexias of undetermined origin.

CONCLUSIONS

The presence of antigen of *Eberthella typhi* has been demonstrated in the serum of typhoid fever patients early in the disease, and a trace of antigen has been demonstrated during relapse. The test is most strongly positive when the somatic "O" agglutinin titer of the patient's serum is low. The test becomes progressively less positive, and finally negative as the titer approaches 1:640. The precipitin test as described is a useful rapid presumptive test for the diagnosis of typhoid fever in inoculated or noninoculated subjects during the first 7 to 10 days of the disease. A positive test clearly indicates typhoid fever; a negative test does not exclude typhoid or the related enteric fevers.

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REMOVING THE SHELL FROM LIVING GRASSHOPPER EGGS

THE newly laid egg of the grasshopper, *Melanoplus differentialis* (Thomas), is covered with a brown, non-chitinous, semi-opaque chorion or shell. After six or seven days' incubation at 25° C. the embryonic serosa, which lies beneath the chorion, begins to secrete a thick, tough, transparent chitinous cuticle over its entire outer surface. As soon as this new membrane has formed it is possible to remove the chorion and the later stages of development may then be followed easily through the glassy-clear chitinous cuticle. Observations are best made with the eggs immersed in water, using either reflected or transmitted light as desired. Formerly it was necessary to remove the chorion from each egg by hand.[1] This was an extremely tedious process and not always successful.

A short time ago it was found that a 3 per cent. (approx.) solution of sodium hypochlorite will dissolve the chorion rapidly and completely with no apparent effect on later development.[2,3] Two minutes' exposure is usually sufficient to remove the entire shell, while the chitinous cuticle remains unchanged. The eggs are watched under the microscope and as soon as the last of the chorion is gone they are transferred at once to water and washed several times.

Eggs treated in the manner just described develop normally and hatch at the same time as do the controls. A series of experiments, involving about 2,000 eggs, was performed to discover how long a period of exposure could be tolerated. Five minutes in the solution had no noticeable effect, but 10 minutes or more resulted in a definite slowing of development, and hatching occurred later than is usual. Many eggs, however, survived even an hour's treatment, and although all these lagged behind the controls more than 50 per cent. of them finally hatched. Thus it is obvious that two minutes' exposure to the reagent is quite harmless.

Since with this simple method any desired number of eggs may be prepared for study with almost no effort and in the time which it formerly took to remove the shells from two or three its usefulness is apparent.

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QUOTATIONS

RESEARCH[1]

PROGRESS in scientific research and development is an indispensable condition to the future welfare and security of the nation. The events of the past few years are both proof and prophecy of what science can do.

Science in this war has worked through thousands of men and women who labored selflessly and, for the most part, anonymously in the laboratories, pilot plants and proving grounds of the nation.

Through them, science, always pushing forward the frontiers of knowledge, forged the new weapons that shortened the war.

Progress in science can not depend alone upon brilliant inspiration or sudden flights of genius. We have recently had a dramatic demonstration of this truth. In peace and in war, progress comes slowly in small new bits, from the unremitting day-by-day labors of thousands of men and women.

No nation can maintain a position of leadership in the world of to-day unless it develops to the full its scientific and technological resources. No government adequately meets its responsibilities unless it generously and intelligently supports and encourages the

[2] Now in charge of typhus investigation, Egyptian State Serum Institute, Cairo.

[1] From President Truman's message to Congress, September 6, 1945.

[1] E. H. Slifer, *Biol. Zentralbl.*, 52: 223, 1932.

[2] Commercial preparations, such as Clorox and Hilex, are satisfactory and easily obtained.

[3] A solution of sodium hypochlorite (Eau de Labarraque) has long been employed by histologists and embryologists to bleach, clean and soften various tissues and tissue products, but its use as an agent for removing the shell from insect eggs which are to be studied alive seems to be new.