course of phetharbital is worth trying, and this may be of value during the exacerbations of jaundice to which these patients are prone, and which may follow alcohol excess or intercurrent infections (Foulk *et al.*, 1959). However, long-term therapy with enzyme-inducing drugs may be harmful, as suggested by the occasional development of osteomalacia in patients with epilepsy, possibly as a result of the induction by anticonvulsants of the hepatic enzymes responsible for the breakdown of vitamin D (Dent *et al.*, 1970). When considering such therapy, even in short courses, for the relief of symptoms in patients with Gilbert's syndrome it would seem reasonable to ensure, by means of a placebo, that any improvement was due to the effect of the inducing drug.

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In-use Evaluation of Safety to Skin of Enzyme-Containing Washing Products

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Summary

The effect on the skin of housewives of using a washing product in the home for all cleaning purposes under conditions of maximal exposure has been studied. Tests conducted on over 4,000 housewives showed that detergents containing proteolytic enzymes had no greater effect on the skin than conventional detergents, even when the hand skin condition was initially poor. The same was true in a further test on 130 housewives with "dishpan" hands. No adverse reactions attributable specifically to the enzyme products were seen. No eruptions from contact with clothes washed in enzyme products were reported from any of the families involved in these tests.

Introduction

Johnson *et al.* (1953) described a test which compared changes in the skin induced by washing products when used at home by matched groups of subjects under controlled conditions. The present report describes five such "home-use tests" conducted in the U.K. on enzyme-containing washing products and an additional test on housewives with hands in poor condition. All were conducted under conditions which maximized the effect of exposure to the products.

Materials

The detergent formulations included both washing and presoak products incorporating 0·3-1·0% of enzyme ingredient (Griffith *et al.*, 1969). Most also contained perborate. The enzyme ingredient contained 5-10% of the pure proteolytic enzyme, a subtilisin of the Carlsberg type (Smith *et al.*, 1968; Keay and Moser, 1969).

Method

The study was designed to compare the primary irritancy of the washing products under test with that of long-established washing products by observing changes in skin condition over the usage period. Such changes are influenced by individual tolerance, by exposure to all exogenous factors in the home environment, and by any protective measures adopted. The groups using each product were made as identical as possible in respect to all variables except the product. Specifically for each usage period the initial mean skin condition was the same for each group. Subjects were asked not to use hand creams or to wear gloves. All the tests except one were performed between November and March. Working-class subjects were investigated, and they were asked to use the products for *all* cleansing purposes, including dish-washing and general household cleaning. All tests were performed under strict double-blind conditions; the products were issued in blank, coded cartons and all gradings made without knowledge of previous observations on that subject or of which product was used.

A cross-over design was used, every subject using two products in succession, each for two weeks. Since for each two-week period the mean initial skin condition of both groups was the same, the final mean condition was a measure of tolerance to the product. Up to five products were evaluated in one test, and these always included at least one well-established non-enzyme product.

EXAMINATION

Objective Assessment.—With a lens (x5) the whole of both hands was assessed for erythema, scaling, and fissuring and a grade assigned on a 10-point scale, in tens. Very slight scaling

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scored 90, pronounced scaling with slight fissuring and redness of knuckles scored 50, and very severe scaling with severe bleeding fissures associated with eczema scored 10. Conditions of intermediate severity were graded accordingly. Other abnormalities unrelated to the above grading were noted separately. All observations in all tests were made by the same observer (R.H.).

Subjective Assessment.—Subjective opinions on the irritancy or otherwise of the product were recorded on a 10-point scale from 10 (very hard on the hands) to 100 (very easy on the hands).

Results

TESTS ON REPRESENTATIVE GROUPS OF HOUSEWIVES

Of the 7,003 housewives participating, 4,119 used enzyme-containing products and 2,884 used non-enzyme controls. In the U.K. tests an average 1·7% did not complete the test; in only one instance was this because an adverse effect on the skin was attributed to the test product, which in this case proved to be a non-enzyme formulation. Some subjects (about 1%) stopped using the products because they thought the products were hard on the skin, but still had their hands examined; this percentage was identical for both enzyme product and non-enzyme product groups.

TABLE I—*Comparison in a Series of Home-use Tests between Average Final Skin Condition Grade of Groups of 300-320 Housewives Using Enzyme Washing Products, and Closely Similar Groups of Housewives Using Non-enzyme Washing Products under Identically the Same Home-use Conditions*

Test No.	Winter Time	Final Condition Skin Grading*				L.S.D.†
		Enzyme Products		Non-enzyme Products		
		No. of Products Tested	Gradings	No. of Products Tested	Gradings	
1	1966-7	2	66·5, 66·8	2	66·5, 66·8	1·1
2	1966-7	3	67·3, 66·4, 65·0	2	61·7, 61·5	1·7
3	1967-8	2	72·4, 70·3	2	71·6, 69·7	1·6
4	1968-9	2	70·2, 67·7	1	65·5	1·8
5	1969-70	3	76·8, 76·6, 75·8	1	74·4	1·4
	Average		70·0		67·5	1·5

* The gradings for each enzyme and each non-enzyme product formulation tested in each test are reported separately—for example, Test No. 2 included three different enzyme formulations and two different non-enzyme formulations.
† Least significant difference for statistical significance between product values (95% confidence level).

Objective Assessment of Primary Irritancy.—The results are summarized in Table I, each figure relating to each product tested. The higher the value, the better the grading and the less irritant the product. Overall, the irritancy of the enzyme products was less than that of the non-enzyme products.

Response of Subjects with Initially Poor Hand Skin.—The data reported in Table I may have concealed differences in response in those subjects whose skin condition was initially below average. Accordingly the data have been compared for the following subgroups of subjects: (*a*) those with "good" hands (grades 80 and above), (*b*) those with "fair" hands (grades 70 and 60), and (*c*) those with "poor" ("dishpan") hands (grades 50 and below). The results are shown in Table II. They have been expressed as the percentages in each group whose grade altered, and show that, on balance, for both types of products the hands initially in good condition got worse while those initially in poor condition improved. All subjects, even those with "dishpan" hands, tolerated enzyme products at least as well as non-enzyme products.

Incidence of Other Abnormalities.—Observations were made on the extent and condition of fissures and of all other skin abnormalities during the studies. The severity and incidence of these was no greater among housewives using enzyme products than among those using non-enzyme products.

TABLE II—*Comparison in a Series of Home-use Tests of the Response of Skin Initially in Good, Fair, and Poor Skin Condition to Exposure to Enzyme Products and to Non-enzyme Products under the Same Home-use Conditions*

Skin Group	Enzyme Products	Non-enzyme Products	Difference
Grade 80-100. Good:			
% Whose grade improved (a)	13	6	
% Who stayed the same	43·5	41	
% Whose grade worsened	43·5	53	
Overall change (a minus b)	−30·5	−47	16·5%
Grade 60-70. Fair:			
% Whose grade improved	32	25	
% Who stayed the same	46	46	
% Whose grade worsened	22	29	
Overall change	+10	− 4	14%
Grade 10-50. Poor:			
% Whose grade improved	62	54	
% Who stayed the same	31	32·5	
% Whose grade worsened	7	13·5	
Overall change	+55	+40·5	14·5%

Subjective Opinion and Observations.—The subjects confirmed the objective findings, with mean scores of 75·3 for the enzyme products against 71·8 for the non-enzyme products (least significant difference = 4·2). There were no reports from housewives participating, or their families, of rashes on the trunk or limbs.

TEST ON HOUSEWIVES WITH BELOW AVERAGE SKIN CONDITION

The analysis of the data in the above series of tests indicated that housewives with poor hand skin condition reacted no differently to enzyme-containing products from housewives with healthier skin. Confirmation of this was sought in a further home-use test in which the subjects were all housewives whose hands had previously been graded 50 or less. Of 253 such housewives who participated, 48% were still graded 50 or less; 21% did regular part-time work as home helps, office cleaners, etc., involving use of detergents, in addition to their household chores; 14% had a long-standing history of skin trouble of one kind or another; and 11% had a history of hay fever, asthma, or skin allergy. The subjects were divided into two groups, one group being asked to use an enzyme-containing washing product for all household cleaning purposes, the others being given a non-enzyme product. Other conditions, including measures to maximize the chances of skin reaction, were as for the normal test described above. Both groups used the test products for five weeks.

Probably reflecting the improvement in weather over the test period, the mean skin grading for the non-enzyme products improved from 54·6 to 61·3 and that for the enzyme products from 54·2 to 63·4. The difference between 61·3 and 63·4 is not statistically significant (least significant difference = 3·0). No adverse reactions attributable specifically to the enzyme product were recorded. The subjects agreed, giving a significantly higher rating to the enzyme product; 71·8 compared with 58·0 for the non-enzyme product (least significant difference 6·9). The most favourable rating recorded was for the enzyme product by the housewives with poor skin (grading 50 and less).

Discussion

Adverse primary irritant reactions thought to be due to enzyme-containing detergents have been described by Jensen (1970a) and by Ducksbury and Dave (1970), though these cases had little in common except that they occurred in a short period in the summer of 1969. Jensen (1970b) has since seen very few cases with similar features and an investigation on five of his original 13 cases showed inconsistent results.

No such cases were encountered in tests covering a wide range of home-usage conditions reported here, or in similar tests among 5,943 U.S. housewives, (Griffith *et al.*, 1969) and 1,995 European housewives (unpublished, Procter and Gamble Ltd.). Further tests are under way and others are planned but the failure to reproduce adverse reactions in the present series of tests, and the lack of confirmatory reports of such cases in the world literature, even though these products have continued to be used by millions of housewives, makes it reasonable to conclude that the addition of enzyme at the low level used in these detergents does not add to their primary irritant potential in use. All the evidence is against enzyme detergents having acted as skin sensitizers in the cases reported (*British Medical Journal*, 1970). Griffith *et al.* (1969) failed to detect any sensitization potential on enzyme products. This suggests that factors other than the enzyme washing product may have been responsible for the outbreaks reported by Jensen and by Ducksbury and Dave.

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Chromosome Breakage and Ultrasound

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Summary

Human lymphocyte cultures were examined for chromosome damage after exposure to ultrasound. Control and treated slides were scored "blind" and showed no evidence of damage due to ultrasound. Neither was there evidence of chromosome damage in blood cultures from six infants whose mothers had ultrasound during pregnancy when compared with that from six infants whose mothers had not. Our results suggest that if diagnostic ultrasound causes chromosome damage it does so with less frequency than acceptable levels of diagnostic *x*-irradiation.

Introduction

Sonar is now an established and invaluable diagnostic tool which has increasing clinical applications particularly in obstetrics and gynaecology. It is therefore of importance to establish that the technique is free from harmful effects to the fetus. Genetic damage is one possible danger, and experience with *x*-irradiation suggests that this danger may to some extent be gauged by examining insonated cells for chromosome breakage. Macintosh and Davey (1970) reported studies on human blood cultures exposed for one and two hours to ultrasound from an ultrasonic fetal heart detector. They found an increase in frequency of chromosome and chromatid aberrations and suggested that clinical ultrasound was potentially mutagenic to the fetus. Their findings prompt us to report an entirely different experience with similar experiments carried out in this hospital in 1966 and referred to briefly elsewhere (Donald, 1969).

Blood cultures consisting of 0·5 ml of whole blood from a healthy adult added to 4·5 ml of Waymouth's tissue culture medium and 0·1 ml of phytohaemagglutinin were incubated at 37°C in sterile polystyrene containers. Two sources of ultrasound were tested seperately in each of three experiments. Source 1 (Doptone I, Smith Kline and French) gave a continuous ultrasound beam at a frequency of 2·0 mHz with an estimated power output at the crystal face of 25-30 mW. Source 2 (Diasonograph, Nuclear Enterprises) gave a pulsed beam of ultrasound at a pulse repetition frequency of 750/sec and was used at maximum output (transmitter attenuation 0 decibels); the transducer frequency was 1·5 mHz. In each case the cultures were placed on top of the ultrasound probe.

Experiments

(1) Cultures were exposed to ultrasound for 13 hours at 20°C and then incubated at 37°C for 72 hours. The container was coupled to the transducer with a layer of oil. Most cells were thus exposed during interphase to at least two cell cycles before harvesting.

(2) Cultures were incubated for 60 hours before exposure to ultrasound for 10 hours at 37°C in a specially constructed water bath, and harvested after exposure to demecolcine without ultrasound for two and a half hours. It was estimated that under these conditions the cell cultures were exposed to at least 60% of the ultrasound energy. The mitoses analysed were therefore derived from cells exposed to ultrasound during most of the cell cycle (including the DNA synthetic phase) before harvesting.

(3) Cultures were incubated for 72 hours at 37°C, demecolcine was then added, and the cultures were exposed to ultra-

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