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Journal of the Society of Cosmetic Chemists

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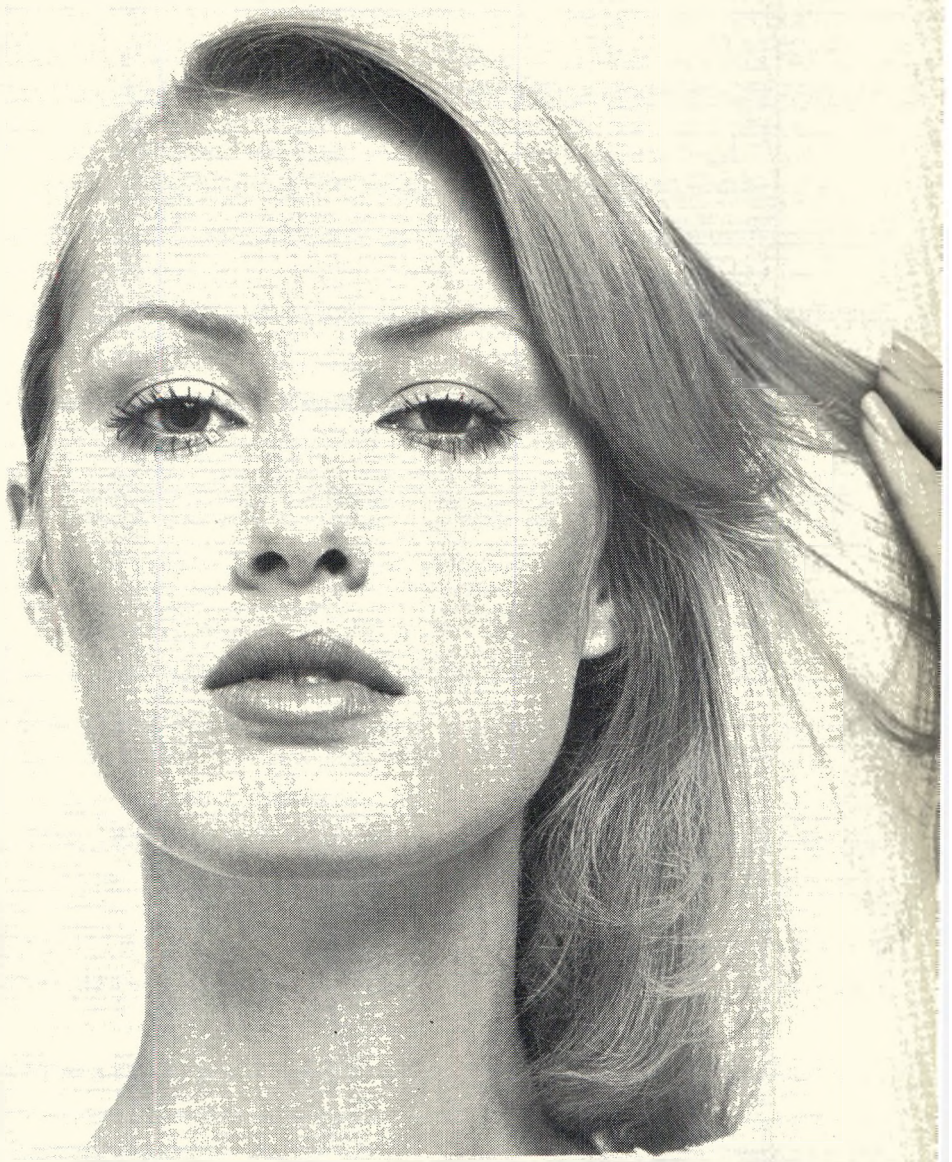

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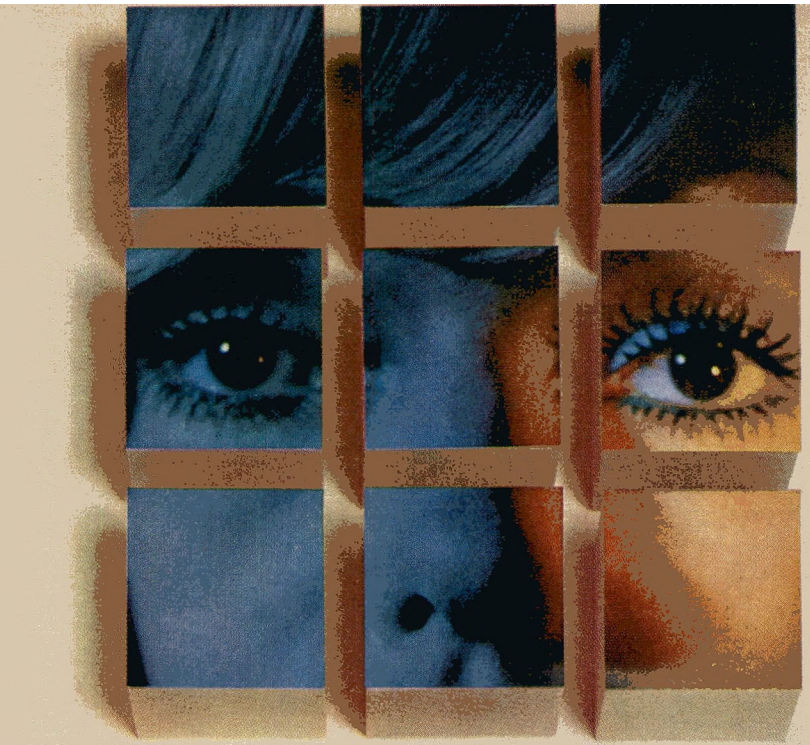

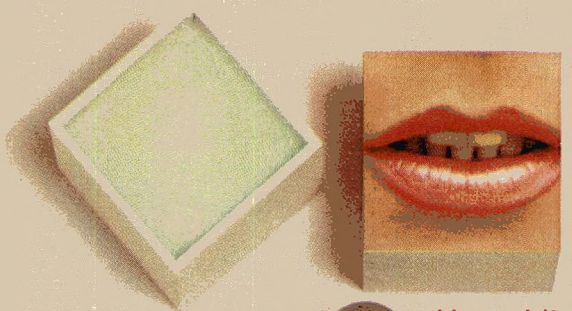

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SYNOPSES FOR CARD INDEXES

The following synopses can be cut out and mounted on 127 × 76 mm index cards for reference without mutilating the pages of the Journal.

Eye irritation tests—an assessment of the maximum delay time for remedial irrigation: R. E. DAVIES, S. R. KYNOCH and M. P. LIGGETT. *Journal of the Society of Cosmetic Chemists* **27** 000–000 (1976)

Synopsis—The rabbit eye irritation test commonly used predictive screening test for assessment of the irritant potential of products that may come into contact with the eye mucosa.

The rabbit eye is more sensitive to irritation than the human eye, and the less effective protective mechanism is partly responsible for this. Irrigation of the rabbit eye after instillation of a test material might reduce its potential irritancy and more nearly approach the human situation, but due to the sensitivity of the rabbit eye it is unrealistic to apply the same time scale that the human may take to implement remedial irrigation.

Results are presented of research carried out to assess the maximum delay time that is acceptable if irrigation of rabbits' eyes with water is to be beneficial in reducing the irritation produced by instillation of sodium lauryl sulphate.

Biological and chemical assay of oestrogenic substances in cosmetics: D. H. LIEM, L. G. HUIS IN 'T VELDE, G. J. RUNDERVOORT, J. ROOSELAAR and J. TEN HAVE. *Journal of the Society of Cosmetic Chemists* **27** 000–000 (1976)

Synopsis—A screening method of testing for oestrogenic activity was developed using direct application of the cosmetic products to the shaven skin of castrated female mice; vaginal smears were taken subsequently. Levels down to 0·00025 oestradiol-17β can be detected. Of the forty-five samples examined, three were shown to contain oestrogenic substances. A quantitative test, based on extraction and subcutaneous administration of the extract to test the animals was developed for further examination of samples found positive in the screening test.

The forty-five samples were also analysed chemically for the presence of the following oestrogenic substances: oestradiol-17β, oestrone, oestriol, and di-ethylstibestrol (DES).

Sex differences in odour perception: E. P. KÖSTER and H. S. KOELEGA. *Journal of the Society of Cosmetic Chemists* **27** 000–000 (1976)

Synopsis—Men and women seem to differ in their sensitivity and variability to, and appreciation of odorous substances. The differences in sensitivity seem to be particularly marked for substances which have a biological significance because they act in the animal world as sexual attractants.

The differences in sensitivity to these substances are probably due to changes in sensitivity which occur in the female population around puberty. Older findings about the variations of the olfactory sensitivity of women during the course of the menstrual cycle also suggested a link between olfactory sensitivity and hormone action, but it is not yet completely clear in which way this interaction takes place.

The following papers have been accepted for publication in the *Journal*:

ORIGINAL SCIENTIFIC PAPERS

The extraction of vinyl chloride from PVC containers
D. A. Tester, B.Sc., Ph.D.

Local infections—experimental aspects
R. R. Marples

The effect of irradiation on packaging materials
F. J. Ley

J. Soc. Cosmet. Chem. **27**, 301–306 (1976)

Eye irritation tests—an assessment of the maximum delay time for remedial irrigation*

R. E. DAVIES, S. R. KYNOCH and M. P. LIGGETT†

Synopsis—The RABBIT EYE IRRITATION TEST commonly used predictive screening test for assessment of the IRRITANT POTENTIAL of products that may come into contact with the EYE MUCOSA.

The rabbit eye is more sensitive to IRRITATION than the human eye, and the less effective protective mechanism is partly responsible for this. Irrigation of the rabbit eye after instillation of a test material might reduce its potential irritancy and more nearly approach the human situation, but due to the sensitivity of the rabbit eye it is unrealistic to apply the same time scale that the human may take to implement remedial IRRIGATION.

Results are presented of research carried out to assess the maximum delay time that is acceptable if irrigation of rabbits' eyes with water is to be beneficial in reducing the irritation produced by instillation of sodium lauryl sulphate.

INTRODUCTION

The rabbit eye has been extensively used for the evaluation of the potential irritancy of cosmetics, toiletries, and household products. The eye of this animal is very sensitive to irritation and corneal damage is readily induced, when compared with the human eye. The differences in the protective mechanisms in the rabbit and human eye are at least partly responsible for the exaggerated response

* Based on the paper presented to the I.F.S.C.C. London, 28 August, 1974.

† Huntingdon Research Centre, Huntingdon, Cambridgeshire PE18 6ES.

in the rabbit eye. In the human, the production of copious amounts of watery tears after an irritant enters the eye, in conjunction with fast blinking helps to dilute and remove the irritant from the eye. The rabbit tear reflex is not as effective as that of the human, and although watery secretions from the tear and Harder's gland, assisted by the action of the eyelids and nictitating membrane, promote the removal of the irritant from the eye, this is neither as rapid nor as efficient as in the human.

There is, therefore, some foundation for the suggestion that irrigation of the rabbit eye after instillation of a test material, might well considerably reduce its potential irritancy, and more nearly approach the human situation.

The present rabbit eye irritation test most widely adopted is that recommended in the U.S.A. Code of Federal Regulations (1). This test has no provision for irrigation of the eye within 24 h of instillation of the test material. Earlier test methods (2) recommended irrigation of the eye with 20 mls of water 2 and 4 s after instillation of the test material, and in 1972 a test (3) was proposed whereby the eyes of three rabbits were irrigated with 300 mls of water 5 min after instillation of the test material.

The difference between 2 s and 5 min clearly indicates a variance of opinion about the length of time that should elapse before irrigation is initiated. Similarly the difference between 20 and 300 mls shows that opinions differ on the amount of water that should be used for this purpose. It was the objective of the research reported here to help clarify those differences of opinion. An attempt was made to find the maximum period of time a particular test material could remain in the eye without causing damage to the cornea, although it is accepted that this time may vary with different materials. Each test was also replicated using quantities of 20 ml and 100 ml of water for irrigation. A well documented anionic surfactant—sodium lauryl sulphate—was used for these initial investigations.

Experimental procedure

New Zealand White strain rabbits in the weight range 2.5–3.5 kg were used in all experiments. The animals were caged separately in metal cages with wire mesh floors and had free access to a pelleted diet (Coney Pellets, 351.B.O.C.M. Silcock) and water at all times.

Before experimentation the eyes of all animals were examined and absence of corneal damage confirmed using a 2% aqueous solution of sodium fluorescein.

A 10% w/v aqueous solution of sodium lauryl sulphate (B.D.H. Limited) was instilled into one eye of each animal by gently pulling the lower lid away from the eyeball to form a cup into which 0.1 ml of the test solution was dropped. The other eye remained untreated and served as a control.

Irrigation of the treated eye was undertaken 4, 10, 20, 30, 60 or 120 s after instillation of the surfactant. Irrigation was by one of two alternative techniques—either 20 ml or 100 ml of lukewarm (37°C) water being used.

The eyes were not irrigated in eleven animals which served as a positive control.

The eyes were examined 1 and 4 h after instillation, and after 1, 2, 3, 4, 7, 14, 21, 28 and 35 days or until there was no visible reaction. The observed reactions were scored numerically according to the system recommended in the Code of Federal Regulations (*Table I*). In addition any dulling of the normal lustre of the cornea was noted.

Table I. Grades for ocular lesions

Cornea	
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal lustre), details of iris clearly visible	(1)*
Easily discernible translucent areas, details of iris slightly obscured	2
Nacreous areas, no details or iris visible, size of pupil barely discernible	3
Completed corneal opacity, iris not discernible	4
Iris	
Normal	0
Markedly deepened folds, congestion, swelling, moderate circumcorneal injection (any of these or combinations of any thereof)	(1)*
No reaction to light, haemorrhage, gross destruction (any or all of these)	2
Conjunctivae	
Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)	
Vessels normal	0
Some vessels definitely injected	1
Diffuse, crimson red, individual vessels not easily discernible	(2)*
Diffuse beefy red	3
Chemosis	
No swelling	0
Any swelling above normal (including nictating membrane)	1
Obvious swelling with partial eversion of lids	(2)*
Swelling with lids about half closed	3
Swelling with lids more than half closed	4

* Bracketed figures indicate lowest grades considered positive under Title 16, Section 1500.42 of the Code of Federal Regulations.

RESULTS

Effects of various exposure times (Table II)

In every rabbit where irrigation of the eye was commenced after a delay of 20 s or longer after instillation of sodium lauryl sulphate, corneal opacity or a dulling of the normal corneal lustre was produced. The reaction observed in the eyes of animals exposed to sodium lauryl sulphate for 20 or 30 s was similar to the effect seen in the eyes of animals where irrigation was delayed for up to 2 min, and only slightly better than that seen in eyes receiving no irrigation at all.

Where the exposure time was reduced to under 20 s, fewer rabbits showed any corneal damage, and where there was corneal involvement, this was less severe, and less persistent (*Table III*).

Only one of twenty-four rabbits developed a corneal opacity when irrigation was initiated within 10 s.

Exposure of the eyes to sodium lauryl sulphate for 4 s did not elicit any opacities, although a dulling of the corneal lustre was seen in four animals.

It may be concluded therefore that the critical exposure time before corneal damage is produced in the rabbit eye after instillation of a 10% sodium lauryl sulphate is in the region of 4–10 s.

Table II. Numbers of rabbits showing corneal damage after instillation of 10% sodium lauryl sulphate

	Delay time before irrigation of eyes (s)						
	4	10	20	30	60	120	No irrigation
No opacity	7	4	—	—	—	—	—
Lack of lustre	4	8	5	7	5	4	2
Opacity grade 1	—	1	3	2	5	4	8
2	—	—	—	1	—	—	1
3	—	—	—	1	—	—	—
Total no. eyes treated	11	13	8	11	10	8	11

Table III. Mean duration of corneal damage and conjunctival irritation after instillation of 10% sodium lauryl sulphate (days)

	Delay time before irrigation of eye after instillation of 10% sodium lauryl sulphate (s)						
	4	10	20	30	60	120	No irrigation
Cornea	<0.5	2	3	8	4	4	4.5
Conjunctiva	4	5	5	9	8	9	9

In addition to corneal damage, considerable conjunctival redness and swelling were produced, even when the eye was irrigated after 4 s. However, the conjunctiva returned to normal much more quickly in rabbits that were exposed to sodium lauryl sulphate for the shorter period of time.

Effects of volume of irrigant

No further reduction in the irritant response was observed when volumes of 100 ml of water were used in preference to 20 ml volumes for irrigation of the eyes. However, inexperienced operators may find some difficulty in adequately rinsing certain test materials from the eye when using only 20 ml, and perhaps the larger amount may be advisable for routine experimentation.

Discussion

Great care is required in the design of laboratory test methods, and in particular it is essential not to lose sight of the objective of the test. Thus, if we decide to irrigate the eye after instillation of a test material we must be sure of our reasons for so doing. For example, we may wish to know if irrigation with water will be beneficial in alleviating the irritation produced by a product accidentally entering the eye of a human being, and how soon after the accident must the treatment begin? If in order to achieve these objectives the rabbit eye is chosen as the 'laboratory model', it must first be accepted that the rabbit eye is different from the human eye. Once this point is accepted it is obviously unreasonable then to apply human standards. Thus to argue that a human being might take 5 min before applying remedial irrigation following the accidental instillation of a product into the eye, and to transpose this time requirement onto the laboratory model, is illogical.

In this paper we have only examined the effects of a single test compound, the anionic surfactant, sodium lauryl sulphate. This material was chosen because of its wide use in eye irritation research and because previously it was a common base material in shampoo formulations. The 10% dilution represented a typical level of surfactant that might enter the eye in a shampoo formulation. Cationic surfactants were not considered, because of their severe effects on the eye mucosa. However, other types of surfactant, especially where there may be differences in the substantive effects on the eye, clearly need to be investigated.

Although individual animals show a wide variation in their response to the same treatment the results obtained in this series of experiments indicate that irrigation must be initiated within 10 s for optimal benefit. The National Academy of Sciences (4) recommended irrigation of some treated eyes 20–30 s after instillation as 'there would seem to be some merit in knowing whether prompt washing would prevent injury in the case of accidental contamination with a household

substance'. This view was supported by the work of Davies and Harper (5) who also recommended the inclusion of a group of animals in the rabbit eye irritation test to investigate the effects of irrigation.

We would endorse these views and recommend that any eye irritation test should be extended to include an additional group of animals to investigate the effects of prompt removal of the test material from the eye. Whilst emphasizing that delay should be as short as practicable, it should also be flexible, and tailored to suit the test compound under investigation. The results obtained in these investigations, although variable, showed that when a 10% solution of sodium lauryl sulphate was instilled into the eye, irrigation must be initiated within 10 s to effect any reduction in the incidence of corneal damage.

Conclusion

In this paper we have attempted to show that the rabbit eye is very sensitive to the introduction of a potential irritant, and any remedial action to remove the irritant from the eye must be prompt.

Corneal damage can be produced within 10 s after instillation of a 10% aqueous solution of sodium lauryl sulphate, and any remedial irrigation must be initiated within this time.

(Received: 21 July 1975)

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(3) Federal Register **37** 83 8534, 28 April (1972).
(4) Principles and procedures for evaluating the toxicity of household substances. Eye irritants. *Natn. Acad. Sci.—Natn. Res. Council Publ.* **1138** 11 (1964).
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J. Soc. Cosmet. Chem. **27**, 307–318 (1976)

Biological and chemical assay of oestrogenic substances in cosmetics

D. H. LIEM,* L. G. HUIS IN 'T VELD,†
G. J. RUNDERVOORT,* J. ROOSELAAR,*
and J. TEN HAVE†

Synopsis—A screening method of testing for OESTROGENIC ACTIVITY was developed using direct application of cosmetic products to the shaven skin of castrated female mice; vaginal smears were taken subsequently. Levels down to 0.00025% OESTRADIOL-17β can be detected. Of forty-five samples examined three were shown to contain oestrogenic substances. A quantitative test, based on extraction and subcutaneous administration of the extract to test animals, was developed for further examination of samples found positve in the screening test. The forty-five samples were also analysed CHEMICALLY for the presence of the following oestrogenic osubstances: oestradiol-17β, OESTRONE OESTRIOL, and DIETHYLSTILBESTROL (DES).

Introduction

Since Zondek's discovery (1) of percutaneous absorption of oestrogenic hormones by human skin and its beneficial effects on wrinkles and skin texture, the cosmetic industry has been interested in the use of hormonal substances in its products. Several papers (2–5) and patents (6, 7) have appeared since then. Other cosmetic effects than anti-wrinkle action have been claimed, such as the development of the female breast (by oestrogens and gestagens (8)) and the depression of abnormal growth of facial and body hair in the female.

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† National Institute of Public Health, Bilthoven, The Netherlands.

The use of hormonal substances is however not without risk. The hazards depend much on the concentration levels of these pharmacologically very active compounds (7). Thus it is important to develop biological and chemical methods to detect the hormonal activity, to identify the active compounds and to determine the levels of concentration. In this paper the methods developed for the detection of oestrogenic substances will be discussed.

The biological methods developed for detection of oestrogenic activity are valid for the female sex hormones (oestradiol-17β, oestrone and oestriol) and other steroidal oestrogens (such as ethinyl-oestradiol) as well as for the stilbene-derivatives (such as diethylstilbestrol (DES) and hexoestrol). Chemical methods, on the contrary, focus the analysis on characteristic chemical structures and thus permit identification of the biologically active compound in many cases. This paper describes biological methods for qualitative and quantitative detection of oestrogenic activity and chemical methods for the detection of oestradiol-17β, oestrone, oestriol and DES.

Methods for the biological and chemical detection of other hormones will be described in a subsequent paper.

Procedures

Sampling

The forty-five samples were purchased from the Dutch market in the second half of 1973. Since no formula is stated on the labels or on the information pamphlets, the selection of the products to be examined was based exclusively on their beautifying or cosmetic claims such as: anti-wrinkle creams for the neck and around the eyes and sometimes for the face, particularly indicated for the older woman, with visible results after about 2 weeks of daily application: growth promoting effect on the female breast; depression of abnormal hair growth in the female, such as hirsutism. Prices of the samples varied from $3 to $40 per unit.

Biological Methods

The Allen-Doisy test (10) was chosen for use with cosmetics because of its specificity. Samples could in principle be administered orally or parenterally (subcutaneous, intravaginal, percutaneous, etc.). In the case of cosmetics, the advantage of percutaneous administration of the specimen without extraction is obvious; however, for a quantitative assay it was necessary to extract the material to be examined and to administer the extract subcutaneously. The percutaneous method proved to be very useful in our hands for screening purposes, as it is simple, sensitive and rapid.

Test animals

Female mice—weight about 20 g—were castrated bilaterally. After 1 week the wounds should have healed and heat phenomena should have disappeared (control with vaginal smear, see below). This was followed by activation with 0.1 ml of 10 μg oestradiol-17β/ml oil solution subcutaneously on three consecutive days; the first sign of oestrogenic action should have appeared about 48 h after the first injection (control by vaginal smear). Animals which did not respond were discarded. This activation was repeated if the animals remained 2 weeks in di-oestrus; a lower concentration of oestradiol-17β (0.5 μg/ml oil) was used in this case. Reactivation was practised in animals which had not been used for testing during the 2 weeks, as well as in animals which had a negative test result. One week after (re)activation the vaginal smear was checked. Immediately after this check (which should be negative), the animals can be used for the actual test. Mice were discarded if they weighed over 35 g.

Administration of samples

For screening, the cosmetic product was applied without extraction, on the shaven skin behind the head (the only place that cannot be scratched or licked by the animal). The samples were applied three times, viz. on the first day at 15.00 and on the second day at 10.00 and 15.00. The sample was applied by brush, which was for one sample only. The application was done as reproducibly as possible, and some control of the quantity applied was possible by backweighing. The mice (three per sample) were housed individually in glass jars with a minimum of sawdust. Although the percutaneous test cannot give exact quantitative results, its excellent sensitivity and its simplicity make it the method of choice for screening.

Extraction of the sample was only practised when a quantitative test was necessary; 1 g of the sample was extracted with 50 ml peroxidefree diethylether. The aqueous layer was discarded. The ether was evaporated and the residue was dissolved in 10 ml 96% ethanol; 0.05, 0.2 and 1.0 ml of this solution were added to 1.5 ml of olive oil. Acetone was added to obtain clear solutions, after which the volatiles were removed under a stream of nitrogen. The remaining oil solution was injected (0.1 ml per animal per injection) at 15.00 (day 1) and at 10.00 and 15.00 (day 2), using 3 animals per concentration. (This was the same scheme as used in the case of percutaneous administration.) Three animals receiving the same treatment could be housed in one cage.

Preparation and staining of the cytological specimen

Vaginal smears were taken by means of a platinum or chromium loop which was adapted to the size of the vaginal opening. The loop was passed through a flame and dipped in distilled water. A droplet of water was placed on an object

glass with the loop; the animal was taken by the tail while gently pressing its back with the third and fourth fingers. The vagina opened itself, so that it was easy to insert the loop. Gently moving the loop back and forth, some vaginal secretion was collected in the loop, which was then dispersed in the droplet on the object glass. One object glass can serve for 9 smears. The preparations were air-dried and fixed for 3 min in methanol. After evaporation of the methanol, the smears were stained in a Giemsa solution diluted 1 : 10, for 20 min, the slides were then rinsed with water and airdried. Microscopical evaluation was done at a medium magnification without oil immersion.

Smears were taken at the following times: Day 3 1500; Day 4 1000 and 1500; Day 5 1000.

The smears were evaluated as follows:

(a) = Basic state as seen in juvenile and castrated animals and in mature intact animals in di-oestrus: over two-thirds of the cell material consisted of leucocytes.

(b and c) = First sign of beginning of oestrus (comparable with pro-oestrus in intact animals): 1/3–2/3 of the cells were epithelic, either with or without a nucleus.

(d) = Distinct oestrogenic activity (comparable with beginning of full oestrus in intact animals): over 2/3 of the cells were epithelic, predominantly without a nucleus.

(e and f) = Positive (comparable with full heat): over 95% of the cells were epithelium cells, either with or without a nucleus.

The highest score per animal and per group of 3 animals was recorded; within one group, reactions should have been comparable; if this was not the case, the test should be repeated.

If the results of a quantitative test was not 'full heat', but rather stage b, c or d, the test should be repeated with a larger amount of material.

Detection limits

Some samples of cream-base were tested percutaneously to which known amounts of various oestrogens had been added. The results of these tests are shown in *Table I*.

The limit of detection at percutaneous application lies under 0.00025% for oestradiol-17β and for diethylstilbestrol; probably the same is true for oestriol (see *Table I*). In the quantitative test, the amounts of extract are chosen to obtain responses on the levels of 0.01, 0.025 and 0.0005% oestrone (or oestradiol-17β-dibenzoate) equivalents per gram. (N.B. 0·1 μg oestrone or 0.1 μg oestradiol-17β-dibenzoate = 1 Mouse Unit.)

Table I. Test for oestrogenic activity in spiked cream samples (percutaneous application)

Oestrogens added	Concentration in (%)	Result of cytological test			Evaluation
None	0.0000	a	a	a	−
Oestradiol-17β	0.00025	e	e/f	c	+
Id.	0.00050	e/f	f	e	+
Id.	0.00100	f	e/f	f	+
Diethylstilbestrol	0.00025	f/d	f/d	f/d	+
Id.	0.00050	f	f	f	+
Id.	0.00100	e/f	f/d	e/f	+
Oestradiol-17β-benzoate	0.00250	f	f	f	+
Id.	0.00350	f	f	f	+
Id.	0.00750	f	f	f	+
Id.	0.01500	f	f	f	+
Diethylstilbestrol	0.00250	f	f	f	+
Id.	0.00350	f	f	f	+
Id.	0.00750	f	f	f	+
Id.	0.01500	f	f	f	+
Oestriol	0.00050	e/f	d	e/f	+
Id.	0.00250	e/f	e	e/f	+
Id.	0.01500	e/f	f	d/e	+

Chemical methods

The proposed chemical methods were focused on four types of oestrogenic compounds, oestradiol-17β and its esters, oestrone, oestriol and the non-steroidal compound DES and its esters. All these compounds have at least one phenolic ring.

Many papers in the clinical and biochemical fields deal with the analysis of these oestrogenic compounds. Lisboa and Dicsfalusy (11) have described the separation and characterization of many steroid-oestrogens with thin layer-chromatography (TLC). Adlercreutz and Luukkainen (12) used gaschromatographic techniques (GLC) for the determination of oestrogens in biological fluids. The most sensitive technique is radioimmunoassay (13). The choice of methods for analysing cosmetics depends very much on the concentration levels of the compounds to be determined. High levels (0.05 or 0.5%) can be easily determined by TLC after a simple extraction procedure. However low levels of oestrogens (*ca* 0.0035%) after the extraction need a cleanup step before the actual chromatographic determination can be carried out. Fortunately the oestrogens are phenols which can easily be purified by the well-known acid/base solvent extraction procedures (14): at pH 1 the phenolic steroids move from the aqueous to the organic phase when shaken with a water-immiscible solvent. At pH 10.5 the phenolic

steroids remain in the organic phase, permitting the removal of a great quantity of interfering compounds. At pH 13 the phenolic steroids moved to the aqueous phase. An alternative cleanup procedure is column chromatography, as described by Jones (15).

For identification TLC is the most convenient and generally applicable method. Visualization in case of samples with low-level oestrogen is however a problem, as too many interfering compounds of the cream matrix respond to the chromogenic sprays. Only in samples containing DES a more specific method of visualization is available (16), namely by u.v. radiation, which converts DES into a yellow phenanthrene derivative, which fluoresces under long-wave u.v. light. For the steroidal oestrogens we find a GLC confirmation very useful. Gas chromatography alone is however not practicable. GLC-data of a natural mixture of (silylated) steroidal compounds clearly shows that GLC alone cannot make a good identification. Combinations of TLC and GLC generally result in reliable results.

Outline of the chemical analysis

The outline of the analysis on *Table II* is mainly based on the information of each product regarding its claimed cosmetic action.

Procedures

Reagents: Acetic acid, acetonitrile p.a., n-hexane, acetone, pentane, toluene p.a., ethanol p.a. (96%), chloroform, ethylacetate p.a., benzene p.a., sodium hydroxide (4M and 0·5M), hydrochloric acid (4M), potassium hydroxide (0·5M in ethanol), sulphuric acid (98%), anhydrous sodium sulphate, sodium chloride, hyflosupercel (filtering aid), alumina for column chromatography (activity II, neutral and activity IV, basic), anisaldehyde, oestradiol-17β-dibenzoate (Merck), oestradiol-17β (Organon and Merck 8964), *N*, O-bis(trimethylsilyl) acetamide, carbon disulphide, oestrone (Merck 8966), oestriol (Organon and Merck 24609 Lab) and diethylstilbestrol (British Drug Houses).

Extraction

Three extraction procedures (A, B and C) are described.

General extraction procedure (A) (0.5 g sample per ml extract). Weigh 2 g of the sample. Add 10 ml of toluene. Distil off the azeotrope toluene/water, in a rotational evaporator under reduced pressure. Add to the dehydrated residue 10 ml of 96% ethanol, 10 ml of acetonitrile and 20 ml of pentane. Shake vigorously and wait until the phases have separated well. Discard the pentane fraction. Evaporate the acetonitrile/ethanol fraction and dissolve the residue in 4 ml of chloroform.

Extraction for low-level 'free' phenolic oestrogens (B) (4 g sample per ml extract). Use 2 ml of extract A. Evaporate solvent. Dissolve residue in 5 ml sodium hydroxide 4M. Shake successively with 2 × 5 ml chloroform and 1 × 5 ml pentane.

Table II. Chemical analytical scheme from oestrogens in cosmetics (based on claimed action of the products)

Action claimed	Possible active hormonal ingredient	Concentration level (%)	Chemical procedures								
			Qualitative				Quantitative				
Anti-wrinkle	DES or DES-esters	0.0035	A†	B	G	H	A	B	G	H	or
							C	E	I		or
							A	B	E	I	
	Steroidal oestrogens:	0.0035	A	B	G	or	A	B	D	I	or
	(a) oestradiol-17β (E2)		A	D	G		C	D	I		
	(b) E2-dibenzoate										
	(c) oestrone										
	(d) oestriol										
Development of female breast	DES or DES-esters	0.1000	A	G*	H		A	B	G*	H	or
		0.2000					A	E	I	or	
							C	E	I		
	Steroidal oestrogens:	0.1000	A	G*	H		A	B	G*	H	or
	(a) oestradiol-17β (E2)	0.2000					A	E	I	or	
	(b) E2-dibenzoate						C	E	I		
	(c) oestrone										
	(d) oestriol										
Depression of abnormal hair growth in the female	DES or DES-esters	0.0100	A	G*	H		A	B	G*	H	or
		0.1000					A	E	I	or	
							C	E	I		

* For extracts with minor background interference on TLC plates, procedure F can be used instead of G.

† For A–I, see text.

Discard organic layers (if necessary after centrifuging). Acidify aqueous layer with hydrochloric acid 4M until pH 1. Shake with 10 ml chloroform. Discard aqueous fraction. Dry with anhydrous sodium sulphate. Evaporate chloroform. Dissolve residue in 0.25 ml chloroform.

Extraction for low-level 'esterified' phenolic oestrogens (c) (4 g sample per ml extract). Mix 1 g of the sample with 20 ml ethanolic potassium hydroxide 0.5M. Boil mixture under reflux for $\frac{1}{2}$ h. Cool, dilute mixture with 30 ml distilled water. Evaporate ethanol on a steambath. Adjust the volume to approximately 25 ml. Add 10 g of sodium chloride, shake vigorously to dissolve as much as possible of the salt. Cool to 10°C and add 0.5 g Hyflosupercel (filtering aid). Filter. Wash residue on filter with 2 × 10 ml sodium hydroxide 0.5M, which has been cooled to 10°C previously. Shake the clear combined filtrates with 2 × 50 ml chloroform. Wait until the separation of the phases is completed (if necessary centrifuge) and discard the chloroform layers. Acidify aqueous layers with hydrochloric acid 4M to pH 1. Shake the phenolic oestrogens with 2 × 25 ml chloroform. Dry with anhydrous sodium sulphate. Evaporate the chloroform solution to 0·25 ml.

Purification of extracts

Two alternative column cleanup procedures were used.

General column cleanup (D). Mix 2 ml of extract A (~1 g sample), 5 g anhydrous sodium sulphate and 3 g Hyflosupercel (filtering aid) to a homogeneous mass. Put this mixture on top of column (diameter 18 mm, glass), filled with 15 g alumina (neutral, activity II according to Brockmann). Elute successively with: fraction I: mixture of 85 ml n-hexane + 15 ml chloroform. Discard this fraction; fraction II: mixture of 50 ml n-hexane + 50 ml chloroform. This fraction will contain: oestradiol-17-dibenzoate; fraction III: 100 ml ethanol 96%. This fraction will contain: oestradiol-17β, oestrone and oestriol.

Column cleanup for diethyl stilbestrol (E). Use 0.25 ml extract B (~1 g sample). Put this on top of a glass column (6 cm × 7 mm), filled with 3.5 g of alumina (basic, activity IV according to Brockmann). Elute successively with: fraction I: 10 ml chloroform. Discard this fraction; fraction II: 5 ml benzene. Discard this fraction; fraction III: mixture of 13.5 ml acetone + 1.5 ml distilled water. This fraction contains DES.

Thin layer chromatography

One-dimensional thin layer chromatography (F). Ready made silica plates without fluorescent indicator, 200 × 200 mm. Solvent 1: n-hexane-ethylacetate (70 : 30). Unlined tank. Solvent 2: *n*-hexane-benzene-ethylacetate (70 : 20 : 10). Unlined tank. Solvent 3: n-hexane-ethylacetate (60 : 40). Unlined tank. Optimal amount of the hormonal substances: 1 μg. Time: approximately 30 min for a 15 cm path. Visualization: mixture of anisaldehyde + sulphuric acid (98%) + acetic acid

(1 : 2 : 97). Spray until wet. Heat at 130°C for 3–5 min, until optimal colours have appeared. For approximate Rf values and characteristic colours see *Table III*.

Table III. Approximate Rf values and characteristic colours

Compound	Approximate Rf values (Solvents) 1	2	3	Colours Initial	Final
(1) Trans-DES	0.38	0.50	0.52	Purple	Purple
(2) Cis-DES	0.35	0.45	0.35	Purple	Purple
(3) Oestradiol-17β	0.18	0.28	0.20	Light green	Ultra-marine-blue
(4) Oestradiol-17β-dibenzoate	0.29	0.32	0.42	Light green	Ultra-marine-blue
(5) Oestrone	0.30	0.35	0.40	Blue	'Berlinblue'
(6) Oestriol	0	0.02	0.04	Grey	Grey

Solvent 1: n-hexane-ethylacetate (70 : 30). Unlined tank.
Solvent 2: n-hexane-benzene-ethylacetate (70 : 20 : 10). Unlined tank.
Solvent 3: n-hexane-ethylacetate (60 : 40). Unlined tank.

Thin layer chromatography (TLC)—two dimensional (G). Silica plates without fluorescent indicator (ready made), 10 × 20 cm. First direction: solvent 3. Second direction: solvent 1. Left section for reference spots.

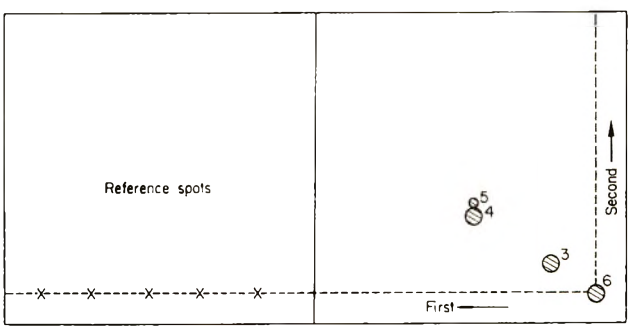

Figure 1. Thin layer chromatography of phenolic steroids. 3 = oestradiol-17β; 4 = oestradiol-17β-dibenzoate; 5 = oestrone; 6 = oestriol.

Thin layer chromatography: visualization of DES (H). This method is very specific for DES. Expose to shortwave u.v. light (e.g. Philips TUV, 15 Watt) for 3 min. DES will be transformed into a yellow phenanthrene derivative, which gives a yellow-brown fluorescence under longwave u.v. light (360 nm).

Gas chromatography (I). The hormonal compounds are first silylated before application to the column. A suitable extract containing ca 50–100 μg of hormonal compound is used. Evaporate solvent. Add 0.2 ml N,O-bis(trimethylsilyl)

Table IV.

Claimed action	No.	Product class	Biological assay				Chemical assay
Anti-wrinkle (for older women)	1	Face cream	a	a	a*	—†	—
	2	Face cream	a	a	a	—	—
	3	Throat cream	a	a	a	—	—
	4	Face cream	a	a	a	—	—
	5	Face cream	a	a	a	—	—
	6	Face cream	a	a	a	—	—
	7	Face cream	a	a	a	—	—
	8	Eye cream	a	a	a	—	—
	9	Face lotion	a	a	a	—	—
	10	Face cream	a	a	a	—	—
	11	Placenta cream	a	a	a	—	—
	12	Eye cream	a	a	a	—	—
	13	Night cream	a	a	a	—	—
	14	Night cream	a	a	a	—	—
	15	Face cream	a	a	a	—	—
	16	Face cream	a	a	a	—	—
	17	Placenta cream	a	a	a	—	—
	18	Face cream	f	b/d	f	+‡	0.0025% DES
	19	Placenta cream	a	a	a	—	—
	20	Throat cream	a	a	a	—	—
	21	Décolleté cream	a	a	a	—	—
	22	Face cream	a	a	a	—	—
	23	Face cream	a	a	a	—	—
	24	Face cream	a	a	a	—	—
	25	Face cream	a	a	a	—	—
	26	Placenta cream	a	a	a	—	—
	27	Beautifying oil	a	a	a	—	—
	28	Face cream	a	a	a	—	—
	29	Face cream	a	a	a	—	—
	30	Royal yelly cream	a	a	a	—	—
	31	Placenta extract oil	a	a	a	—	—
	32	Idem aqueous	a	a	a	—	—
	33	Face cream	a	a	a	—	—
	34	Face mask	a	a	a	—	—
	35	Face cream	a	a	a	—	—
	36	Face cream	a	a	a	—	—
	37	Face cream	a	a	a	—	—
	38	Face cream	a	a	a	—	—
	39	Face cream	a	a	a	—	—
Development of the female breast	40	Bust cream	a	a	a	—	—
	41	Paper plaids	a	a	a	—	—
	42	Paper plaids	a	a	a	—	—
Depression of abnormal hairgrowth in the female	43	Body cream	f	f	f	+	0.1% DES
	44	Body cream-mild	f	f	f	+	0.01% DES
	45	Body cream	a	a	a	—	—

* Cytological evaluation (see preparation and staining of cytological specimen).
† — =Oestrogenic action absent; + = oestrogenic activity present.
‡ Results of quantitative biological assay see *Table V.*

acetamide (BSA). Heat at 50°C for 10 min. Blow off excess BSA. Dissolve in 250 µl carbon disulphide. Inject *ca* 3 µl on the following. Glass column: 5% SE-30 (or 5% OV-17) on Chromosorb G. 1500 mm × 2 mm internal diameter. Temperature of column for SE-30: 190°C and for OV-17: 230°C. Flame ionization detection. Detector FID, 240°C. Carrier gas nitrogen: 30 ml/min.

Results and discussion

The results of biological and chemical assay for oestrogens of 45 market samples of cosmetic products, which have been purchased in the Netherlands in the second half of 1973 are tabulated in *Table IV*.

Of thirty-nine anti-wrinkle preparations (including preparations containing placental extract) only one (18) contained detectable oestrogenic activity on investigation with the biological screening method. This finding was confirmed by the chemical methods.* From these data it can be concluded that oestrogenic substances were not present generally in anti-wrinkle cosmetics on the Dutch market.

Sample 18 has been evaluated quantitatively with the quantitative biological method. (see *Table V*). The biological data (100–200 Mouse Units per g, which corresponds to 0.0010–0.0020% oestrone equivalents) were in fair agreement with the chemical findings (0.0025% DES), as the biological activity of DES,

Table V. Quantitative biological assay of sample 18

Dosage per animal (~ mg sample)	Cytology of vaginal smears						Result
5*	a/b	f/d	a/c				±
10	e/f	e	e				+
10	d/e	d/e	e				+
20	f	f	f/d				+
40	e/f	e/f	e/f				+
100	f/f	f	f				+
0.075 µg oestrone	e/f	e/f	e/f	b/c	c	.	+
0.15 µg oestrone	f	e	f	f	f	e	+

*Corresponds to 0.5 to 1 Mouse Unit (0.05–0.10 µg oestrone).

Conclusion: 1 g of sample 18 contains 100–200 Mouse Units (equivalent to 0.001–0.002% oestrone).

N.B. Sample 18 has been assayed chemically; results 0.0025% diethylstilbestrol.

* Some other hormonal, non-oestrogenic substances with presumed anti-wrinkle action, as ethisterone and pregnenolone, have been identified and determined in several samples. The analysis will however be described in a subsequent paper.

when administered subcutaneously in oil solution, was shown to be approximately equal to that of oestrone (Huis in 't Veld, unpublished data).

The three samples for promotion of female breast development (samples 40, 41 and 42) lacked detectable oestrogenic activity.

Of three samples for depression of abnormal growth of hair in the female (samples 43, 44 and 45), two (samples 43, 44) 'Hairstop' cream, both of the same brand, showed considerable oestrogenic activity, which has been confirmed by the results of chemical analysis.

ACKNOWLEDGMENT

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Sex differences in odour perception

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Synopsis—Men and women seem to differ in their SENSITIVITY and VARIABILITY to, and appreciation of, odorous substances. The differences in sensitivity seem to be particularly marked for substances which have a biological significance because they act in the animal world as SEXUAL ATTRACTANTS.

The differences in sensitivity to these substances are probably due to changes in sensitivity which occur in the female population around puberty. Older findings about the variations of the olfactory sensitivity of women during the course of the MENSTRUAL CYCLE also suggested a link between OLFACTORY SENSITIVITY and HORMONE action, but it is not yet completely clear in which way this interaction takes place.

Introduction

Odours and the sense of smell play an important part in the sexual attraction and the sexual behaviour of many animal species. Whether olfactory communication is also an important factor in the sexual behaviour of man is still a completely open question. In recent years, a large amount of money and effort has been put into the search for a possible human sex attractant or 'pheromone', but little has come out of this so far.

The research on sex differences in odour perception reported here has different and much older origins and is only indirectly relevant to the question of the existence of a human pheromone. The question asked here is simply whether men and women differ in their perception of odours and if so, what possible mechanisms might be responsible for the differences in odour perception found. Whether or not such differences may also have a functional meaning in human sexual behaviour cannot be decided on the basis of these experiments alone.

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Three kinds of differences in odour perception between men and women will be discussed: (a) differences in sensitivity to low concentrations of odours; (b) differences in variability of the sensitivity to odours; (c) differences in the qualitative appreciation of odours.

Differences in olfactory sensitivity

In 1899 Toulouse and Vaschide (1) reported on the already conflicting results of research workers concerning differences in olfactory sensitivity between men and women.

Since then there have been a number of authors who found no such differences but others, often using more refined techniques and more carefully selected odorous substances, reported that women were often more sensitive to odours than men. However, even today there is conflicting evidence.

Le Magnen (2) used among other substances a musk-like odour called 'exaltolide' (cyclo-pentadecanolide) and found that for this substance the sex difference was rather large. According to him adult women are very sensitive to cyclo-pentadecanolide whereas young children and adult men do not perceive the odour at all, or find it very weak. Le Magnen (2) claimed that these differences were specific for the sensitivity to cyclo-pentadecanolide and urinoid odours like pyridine. On the basis of these findings he formulated the hypothesis that the sensitivity of these 'biologically significant' odours is determined by sex hormones. Oestrogens would improve sensitivity to them whereas androgens would diminish the sensitivity to the biologically significant odours but in contrast would improve the sensitivity to other odours like safrol, phenol etc.

Le Magnen (3) reported that in rats, androgens seem to develop the sensitivity to the musky odour to which adult male rats are the most sensitive. Pietras and Moulton (4) also found that male rats are, on the average, more sensitive than female rats, not only for exaltolide, but also for 'neutral' odours. Le Magnen's (2) '*specific*' hypothesis stands in contrast to a more '*general*' hypothesis formulated by Broverman *et al.* (5). Broverman (5) supposed that sex differences in cognitive tasks, conditioning and in sensory thresholds are reflections of the balance between the activating influence of central adrenergic processes and the inhibitory influence of central cholinergic processes, which in turn, are influenced by the gonadal steroid sex hormones, androgens and oestrogens. Since the oestrogens are more potent activating agents than the androgens, females are more activated, or less inhibited than males. This would manifest itself also in a higher feminine sensory sensitivity.

Koelega (6); Koelega and Köster (7) tested these hypotheses. The olfactory thresholds of adult men and women and of children before and during puberty were determined. A number of different substances both from a 'biologically

neutral' group (e.g. amyl acetate) and from a 'biologically significant' group, like cyclo-pentadecanolide and 11-oxahexadecanolide (Musk R-1), were used.

The results showed that whereas there was a slight sex difference for amyl acetate in the young children (average age about 9.0 years) and in the adult group (average age 20 years), a much more marked sex difference was found for adults, but not for children, with cyclo-pentadecanolide and 11-oxahexadecanolide. For these substances the sensitivity of young children and of children in puberty (average age 16.7 years) did not seem to deviate significantly from that of adult men, but the threshold of adult women was significantly lower (reflecting a higher sensitivity).

This is illustrated in *Fig. 1*, which gives the results of six groups of subjects. The results of the male groups do not seem to be influenced by age, but the results of the adult female group lie clearly above those of the other groups indicating their detection (expressed in probits or z-scores) of the same concentrations is better.

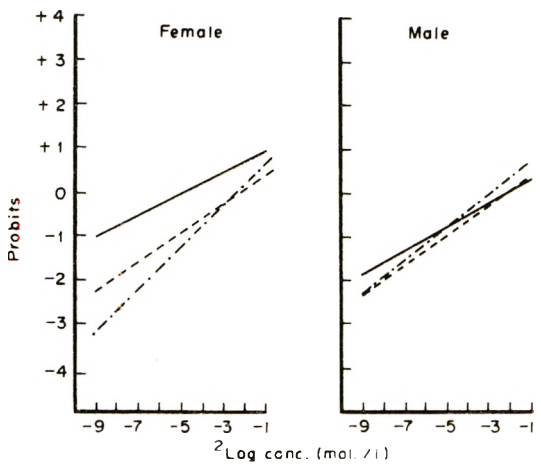

Figure 1. Relationship between detection probability in z-scores and the logarithm of concentration for cyclopentadecanolide in three different age groups. Each of the subjects participated three times. (Female:—N=58 $\bar{M}$ age 20.1 Y=0·24X+1.18; · — · N=43 $\bar{M}$ age=16.7 Y=0·47X+1.09; - - - N=32 $\bar{M}$ age=10.0 Y=0.33X +0.66. Male:—N=52 $\bar{M}$ age=20.4 Y=0.27X+0.54; ·—· N=48 $\bar{M}$ age=16.7 Y=0.37X+1.04; - - - N=37 $\bar{M}$ age=9.7 Y=0.33X +0.62.)

Koelega and Köster (7) found also striking sex differences between adult groups for androstenone and a number of related substances, which can also be characterized as 'biologically significant'. Again women were more sensitive than

men. This was also true for a 'neutral' odour like m-xylene but here again the difference was much less marked.

According to some authors the incidence of a specific anosmia, i.e. total incapacity to perceive a specific odour, is much more widely spread among adult men than among adult women. Le Magnen (2) reported that about 50% of the adult males show an anosmia for cyclopentadecanolide. Griffiths and Patterson (8) found that 7.6% of the women in their experiment were unable to detect the odour of androstenone, in contrast to 44·3% of the men. However, Whissell-Buechy and Amoore (9) found no sex difference in the incidence of pentadecalactone anosmia. They found significant differences in the frequencies of specific anosmias between two human races: the musk anosmia occurred in about 7% of the Caucasians but in none of the Negroes, whereas the anosmia for isovaleric acid was prevalent (about 9%) among Negroes and uncommon (about 1%) in Caucasians. The inability to smell pentadecalactone seems to be inherited as a simple recessive autosomal character. Comfort (10) pointed out that these results raise several points of biological interest, as both isovaleric acid and musks are candidates for consideration as functional pheromones, the former at any rate in monkeys. Koelega and Köster (7) were not able to show occurrence of a specific anosmia for musk or androstenone *in a systematic way*. Sometimes a person who could not smell the highest concentration presented to him (or her) in a particular session of the experiment, did quite well on other occasions.

Much seems to depend on the method of stimulus presentation used and on instructions.

The general hypothesis forwarded by Broverman *et al* (5) cannot explain why the differences found in the adult groups are much more pronounced for the biologically meaningful odours than for the other odours and why a sex difference for amyl acetate should be found at an early age when the sex hormones are not yet active. Differences in motivation may have contributed to this difference among young subjects, although Koelega (7) tried to hold motivation constant by the prospect of rewards for the best smellers.

The specific hypothesis of Le Magnen (2) is supported to some extent by these results since the sensitivity for the biologically significant odours seems to develop well only under the influence of the female sex hormones; adult women are clearly more sensitive than men for these odours.

However, Le Magnen's (2) hypothesis also suggests a greater sensitivity in men than in women for biologically non-significant odours like amyl acetate and m-xylene. The results of the experiments do not support this.

In fact, women do slightly better on these odours too. Motivational differences and differential smoking habits might contribute to this result.

DIFFERENCES IN VARIABILITY OF THE SENSITIVITY

Although women are in general perhaps more sensitive to odours than men, they also show larger variations in their sensitivity.

These variations seem to be linked with variations in sex hormone production. Le Magnen (2) was the first to demonstrate that for exaltolide there existed a relationship between the olfactory sensitivity in women and the course of their ovulatory cycle. During menstruation the sensitivity is at its lowest. Shortly afterwards the sensitivity goes up to a maximum situated in time around or just before ovulation. Then there is a rather sharp fall in sensitivity to a level just above the one found at menstruation. This level is maintained until the onset of menstruation.

Vierling and Rock (11) confirmed Le Magnen's (2) finding with exaltolide, however, they found two increases in sensitivity: one just before the theoretical ovulation (17 days before menstruation) and one during the luteal phase (8 days before menstruation). Le Magnen (2) had also found such a second peak in acuity in a woman with a long cycle.

In line with this specific hypothesis Le Magnen (2) supposed that his result would be specific for exaltolide and other 'biologically significant' odours. He used several other substances, but the curves obtained for these substances show very little variation over the same period.

Meixner (12) also confirmed Le Magnen's (2) result with exaltolide but found that the acuity for pyridine, a urinoid odour according to Le Magnen (2), did not parallel the fluctuations found for exaltolide but was probably related to the phases of the menstrual cycle, whereas sensitivity to two neutral odours, although varying together, was not related to the menstrual cycle. The results indicated specific rather than generalized changes in olfactory sensitivity during the female sexual cycle.

Kahn (13) obtained inconsistent data for three neutral odours, but the number of measurements in this experiment was small.

Schneider and Wolf (14), using a neutral odour (citral) as a stimulus found a decrease in the sensitivity during menstruation, but they did not find the marked increase of the sensitivity around ovulation. Since the decrease in sensitivity during menstruation may well have been an artefact resulting from lack of motivation during that period, Le Magnen's (2) claim of the specificity of the effect was not seriously challenged by these results. However, in a number of experiments, Köster (15, 16) was able to show that similar variations of the olfactory sensitivity with the ovulatory cycle could be found for another 'neutral' odour (m-xylene), although the variations were definitely less pronounced than in Le Magnen's (2) study. He also found that the variations observed are dependent upon ovulatory cycle duration. Women with short (28 days or somewhat less) cycles behave exactly in the way described by Le Magnen (2), but women with long cycles behave in the opposite way. This has been confirmed in two separate experiments

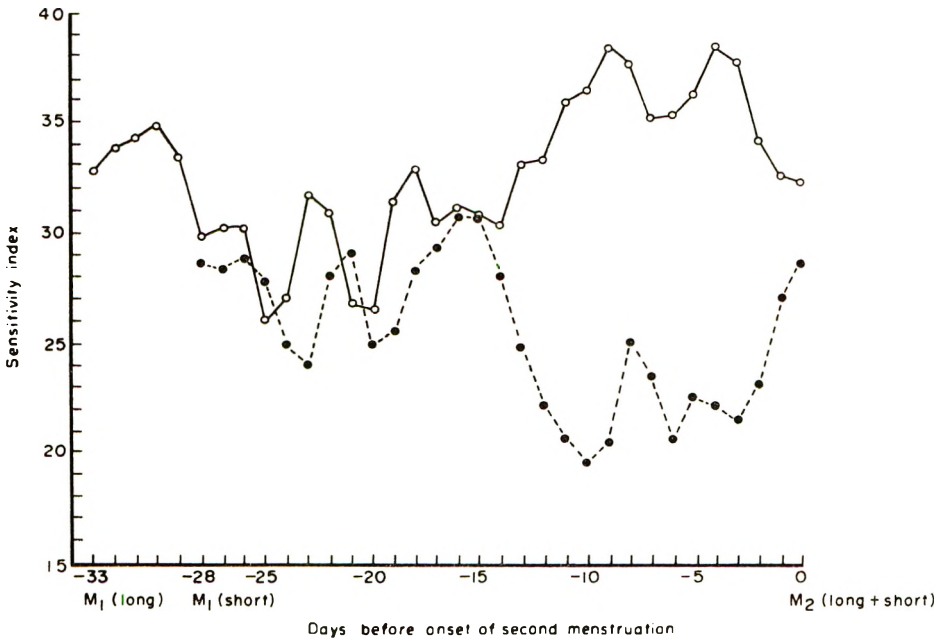

Figure 2. Relationship between menstrual cycle and olfactory sensitivity. Average sensitivity indices per day for two groups of different cycle length. ○—○ long cycles; ●- - -● short cycles.

with two independent groups of women. The difference between the long and short cycle groups is illustrated in *Fig. 2.*

For both groups there is a transition point which lies about 14 days before menstruation. In the short cycle group this is also the point of greatest sensitivity reached in the entire cycle. After this day the sensitivity of the short cycle group decreases drastically as in Le Magnen's (2) experiments.

For the long cycle group, however, the period of high sensitivity starts at the transition point and lies between this point and the next menstruation. The results obtained in these experiments showed clearly that there can be found differences in sensitivity with the course of the ovulatory cycle for a 'neutral' odour like m-xylene.

It should be noted that the variations found for m-xylene are considerably smaller than those found by Le Magnen (2) for exaltolide. Also, the relationship between the sensitivity and the ovulatory cycle seems to be more complex than was expected as is illustrated in the results obtained with different cycle durations.

Pietras and Moulton (4), using a behavioural method, found cyclic variations in the sensitivity of female rats to a number of different substances (cyclopentanenone, eugenol, a-ionone and exaltolide). Sensitivity was maximal around ovulation.

Amoore *et al.* (17) failed to demonstrate cyclic changes in women's sensitivity to the odour of pentadecalactone (Thibetolide), but Schiffman (18) found an increased sensitivity at the eleventh day of the cycle for cyclopentadecanone. Good *et al.* (19) with a signal detection procedure also confirmed Le Magnen's (2) findings and found further indications of a hormonal influence on olfactory sensitivity with pregnant and hypogonadal subjects.

Differences in the qualitative appreciation of odours

For most odours men and women do not differ in their appreciation in a systematic way. However, there are some odours in the 'biologically significant' group which seem to smell differently to men and women. Moncrieff (20) investigated odour preferences within a group of ten odours with a large number of subjects. Men and women did not differ of opinion when very agreeable or very bad odours were concerned. Men preferred the smell of musklactone more than women did. This difference in preference seems to develop around puberty and is less outspoken after the age of 40, because after that age the odour is much more preferred by women than before it. Men seem to be more stable in their preferences through life than women (see *Fig. 3*).

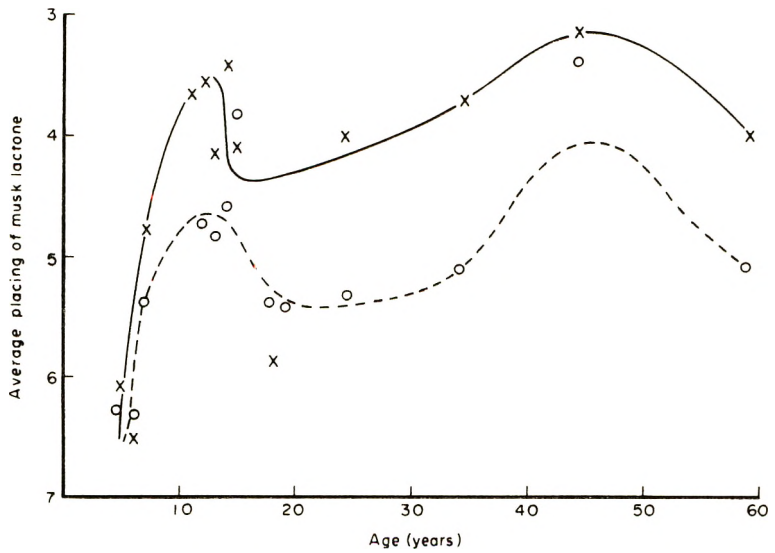

Figure 3. Relationship between age and preference for the odour of musk lactone (from Moncrieff (18)). ×—× males; ○- - -○ females.

One group of substances, androstenone and related substances, demands special attention here, since these odours seem to be perceived very differently by different people.

Beets and Theimer (21) asked a number of men and women to characterize three of these substances, including androstenone itself, with a set of predetermined adjectives. Women found the odours to be more often sweaty than men. Men in turn used the terms animal and sexual more often. Both groups qualified the odours most frequently as a urine odour.

Differences in these kinds of experiments may be due in part to experience and socio-cultural factors like a female hesitance to use the term 'sexual'. Furthermore the state of health of the subject and his absolute threshold, which for the sense of taste was found to be correlated with preferences, may play a role (Kaplan (22)). In some studies differences in preference might be due to differences in sensitivity.

Griffiths and Patterson (8) using 5a-androst-16-en-3-one found that women judged the smell of it significantly more unpleasant. Furthermore they found that the more sensitive a subject was the greater was his or her dislike of the odour, but within groups with the same threshold women disliked the odour more than did men.

Fischer (23) also found that men liked the odour of a natural musk more than women did.

Concluding remarks

In all cases in which sex differences are found these differences seem to be more pronounced for substances like musks which are used as sexual attractants in the animal world or for substances like androstenone and related steroids which are also clearly sex-linked. Male odours are usually characterized as musky, which was corroborated recently by Russell (24) who found that the sex of a person can be determined by the odour of his clothing, and female odours are characterized as sweet.

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Book review

PATCH TESTING GUIDELINES

K. E. Malten, Catholic University of Nijmegen; J. P. Nater, State University of Groningen; W. G. van Ketel, Free University of Amsterdam. (1976) Dekker and van de Vegt, Nijmegen, the Netherlands. Paperback Dfl 29.5.

This is an exceedingly practical paperback written by a group of authors who probably have as much experience as anyone in the field. They provide a broad selection of hints and tips, mainly of empirical origin rather than academic. Obviously this is indispensable for those called upon to carry out patch-testing of the type discussed but the precise aims of the testing are important when considering its relevance for those without a direct interest in the management of skin patients.

Professor Malten and his co-authors deal with the technique of patch-testing essentially for diagnostic purposes. Cosmetic scientists are more often concerned with predictive (so-called 'prophetic') testing and the differences are perhaps worth reiterating. Patch-testing may be used to study both irritation and allergic skin response or sensitization, but in the present context, we are only concerned with the latter. A dermatologist is usually seeking to determine the cause of allergic contact dermatitis in a particular patient. By contrast, the cosmetic scientist wants to know whether a raw material or a formulation has an abnormally high propensity to elicit sensitization responses in a large population of users.

One of the striking differences between these approaches mentioned in the book, is seldom appreciated except by dermatologists. This interesting fact is that an individual may become sensitized to a particular compound without showing any clinical signs of qualitatively or quantitatively altered skin condition, i.e. without developing an allergic contact dermatitis. Indeed a patient with a skin condition elicited by one compound may also be capable of giving positive patch-test responses to several others, although exposure to these has not so far resulted in manifest skin problems. So, in treating the individual patient, the physician is primarily concerned to find the particular substance(s) to which his skin is actually responding; on the other hand, the cosmetic research worker wants to know the overall sensitizing capacity of his test material, whether or not in particular individuals any skin disorder is provoked. Hence approaches suitable for clinical practice and cosmetic research are related but not the same.

An illustration of the practical merit of the book is shown in the advice to use square patches, since irritant responses are usually confined to the original shape (and size) of the patch whereas sensitization mostly leads to an extending reaction, often rounded. The distinction would be missed if circular patches are used. Such advice ought not to be dismissed as merely anecdotal; the authors, in the course of carrying out many thousands of patch tests, have accumulated a wealth of experience which is now made available to everyone.

Rather less than half the pages are devoted to methodology, the remainder being mainly concerned with the current 'standard tray' of twenty relatively common sensitizers listed by the International Contact Dermatitis Research Group and going into considerable detail to show possible sources of exposure to these and chemically-related substances. In treating an individual patient, the demonstration of a positive patch test response is only part of the story; the next stage is to identify opportunities for exposure to the allergen and to show that avoidance of contact leads to relief of the skin condition . . . only then, incidentally, may the correctness of the patch-test diagnosis be taken as confirmed.

The snag is that a standard array of common sensitizers becomes outdated fairly quickly. So do references to uses of chemicals included in the tray. This part of the book cannot therefore retain its usefulness indefinitely but presumably revised editions will follow when necessary.

Most of the contents insofar as they relate to the problems of individual patients, will not be directly applicable to the cosmetic scientist's work. Good advice abounds nevertheless, e.g. suggestions for non-irritating, non-sensitizing solvents for general patch-testing use. One of our main problems is not answered, namely what action to take, if any, following the demonstration of positive allergic patch-test responses to our formulations in one or two patients. Should we regard these patients as odd and forget about them or should the formulation be deemed obnoxious and in need of alteration? A book such as the one now being reviewed will at least provide some of the underlying knowledge on the basis of which we may attempt to draw rational conclusions.

N. G. VAN ABBÉ

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Society of Cosmetic Chemists of Great Britain 1976 Medal Lecture

The 1976 medal of the Society of Cosmetic Chemists of Great Britain was presented to Dr C. D. Calnan at a meeting held at the Royal Society of Arts, on Thursday 4 March 1976.

Dr Calnan who is Consultant Dermatologist at St John's Hospital for Diseases of the Skin went on to present his medal address 'Dermatocosmetic relations'.

Dr C. D. Calnan (left) receiving the 1976 Silver Medal of the Society of Cosmetic Chemists of Great Britain from Dr F. G. Brown, President of the Society.

In presenting the medal the President of the Society Dr F. G. Brown emphasized the pleasure it gave him to honour a member who has contributed so much to our knowledge of the dermatological effects of cosmetics and perfumes.

Dr Calnan's address which emphasized the need for a meaningful relationship between cosmetic chemists and dermatologists was followed by a note of thanks proposed by the Vice-President Mr D. F. Williams.

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