J. Physiol. (1955) 130, 543–558

THE SORPTIVE PROPERTIES OF THE OLFACTORY MEMBRANE

By R. W. MONCRIEFF

From The Consulting Laboratory, 83 St Pancras, Chichester

(*Received* 18 *April* 1955)

The view that olfactory discrimination is made possible by selectivity of adsorption characteristics of the olfactory receptors for different odorants was recently put forward by the author (Moncrieff, 1954). It was shown that within the limits of a small group of odorant materials examined, those substances that had different odours did behave differently towards such adsorbents as activated carbon and silica gel, whereas those substances which had odours of the same general type, e.g. fruity, behaved not very differently amongst themselves to the adsorbents. A comparison was made also of the adsorption behaviour of compounds of very similar odour but of very unlike chemical constitution, and the findings indicated that the adsorption behaviour was related to the smell and was not obviously related to the chemical constitution. The underlying argument was that if olfaction is, indeed, stimulated by adsorption and if discrimination of smell is due to selectivity of adsorption on the olfactory membrane, then substances which smell differently would probably be easily but differentially adsorbed on the common inorganic adsorbent materials, whilst substances which smelt more or less the same would behave fairly similarly as a group towards different adsorbents. That, in fact, such behaviour was observed supported not only the view that olfactory discrimination was due to selectivity of adsorption, but also the more fundamental postulate that the primary stimulus for olfaction was an adsorption process. This paper is concerned with describing some direct evidence that odorant materials are adsorbed by the interior nasal surfaces and especially by the olfactory membrane, and also with an examination of how the view that olfaction is stimulated by adsorption of the odorant on the olfactory membrane accords with some of the properties of smell.

METHODS

Three methods were used.

(1) *Passing odorous air through adsorbent columns*

This method was similar in principle to, and the apparatus was exactly the same as, that described earlier (Moncrieff, 1954); the only essential difference was that whereas, previously, inorganic adsorbents such as active carbon or silica gel had been used to pack the adsorbent tube, now chopped tissue from the olfactory regions of the cleft heads of slaughtered bullocks and sheep was used to pack the adsorbent tube. This tissue was removed from the upper turbinates in the pigmented area and cut into small pieces which approximated to 2–3 mm cubes; a vertical glass tube (0·5 cm^2 internal cross-section) was packed to a height of 10 cm with the cut material in such a way that flow of air through it would not be inhibited. Then air which had been odorized by having been passed over benzaldehyde was passed from the bottom up the vertical adsorption tube at a rate of flow of 10 c.c./sec and the nose was applied to the outlet end of the adsorption tube. At the time that experiments with this method were made, the animals had been dead for from 2 to 4 hr. In a few experiments tissue similarly cut from a rabbit's head was used.

(2) *Deodorization of small fixed volumes of air*

This method consisted of collecting, in two wide-neck bottles, air that had been odorized by passing over some such odorant as benzaldehyde or ethyl acetate; this odorized air was delivered at a rate of 10 c.c./sec for 10 sec and the bottles were then quickly covered. Then into one of the bottles a part of the pigmented turbinate from one side of a sheep's head was introduced. The rates of disappearance of the odour of benzaldehyde (or other odorant) in the two bottles were compared, to ascertain the effect of the piece of olfactory tissue.

In a modification of this method two wide-neck bottles were half filled with freshly cut lawn mowings which had a sweet vernal smell; as before, to one of the bottles a portion of pigmented turbinate was added and the rates of disappearance of the grass odour in the two bottles were compared. The experiment with grass was the only one in which the odorant material was directly in contact with the olfactory tissue; in all other experiments the air was blown over a relatively powerful odorant (much more powerful than grass), and was collected in two bottles, to one of which a portion of the sheep's olfactory membrane was added. The smell in each of the bottles was then observed at intervals.

Later, in order to determine whether the sorption of the odorant from the air, which was observed in the early experiments, was taking place over the whole area of the interior nasal surfaces or whether it was confined to one area, tests were made with tissue from different parts of the nose. A small piece of tissue was cut from four places:

(1) The chin of the sheep (about 0·5 g).

(2) The anterior turbinate at a distance of about 5 cm from the snout (about 2 cm^2).

(3) The anterior turbinate at a distance of about 10 cm from the snout (about 2 cm^2).

(4) The pigmented region in the upper posterior turbinate system (about 0·5 cm^2).

The four pieces of tissue were introduced into four 600 ml. beakers which were then supplied with odorized air for 12 sec at 18 c.c./sec and then immediately covered. Sniffs were taken by two observers at frequent intervals and the time taken for the odour of benzaldehyde to disappear was noted. In control experiments, beakers that did not contain tissue were used and also beakers that did contain active carbon, a known adsorbent, so that a comparison of behaviour could be made. Observations were made with two different odorants, benzaldehyde and ethyl acetate.

(3) *Flow of odorized air through whole head (sheep)*

In this third method, odorized air was passed through the nasal passages of a sheep's head and the appearance of odour, whether at once or after an observed interval of time, in the air issuing from the head was noted. The technique adopted was as follows:

The head of a newly killed Suffolk lamb of about 6 months was obtained. The orifices in the head were then sealed; corks were inserted from the cut neck into the gullet and windpipe and

plenty of molten beeswax was poured over them; then sealing wax was dripped on to the mouth; a right-angled glass tube (3 mm) over which a cork was pushed was inserted into each of the nostrils so that the glass tube projected about 3 cm into the nose, and so that the cork filled most of the opening to the nostril. Sealing wax was dripped on the corks to make a good seal; as little wax as possible was used on the nostrils, so that if the glass tube was accidentally moved in the subsequent experiments, the small part of the nostril would move with it and the tube would not break away. The first head was devoted to practising this sealing, the test being to blow air up the glass tube projecting into one nostril and to observe its appearance down the other tube, and nowhere else, when the head was completely inmersed in water. The immersion of the first head was carried out simply to ensure that the sealing method was satisfactory; this head was not used for smell tests, nor were later heads that were used for smell tests immersed in water.

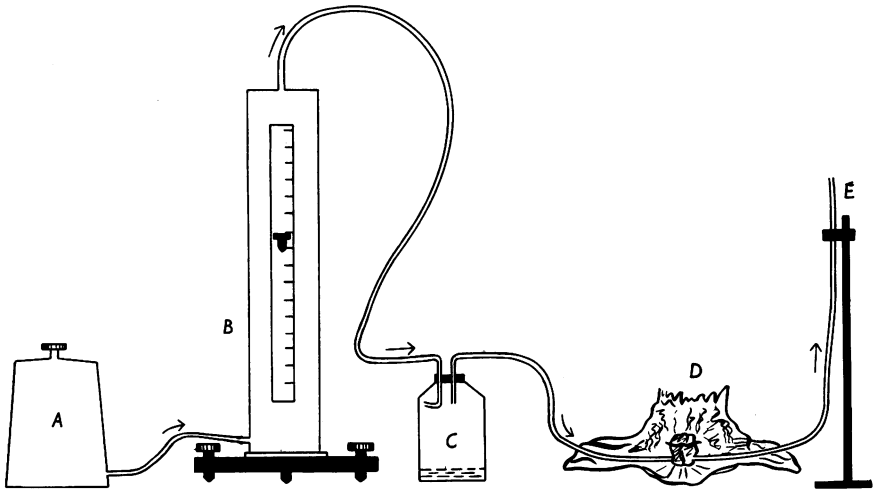

Fig. 1. Diagrammatic representation of apparatus used to pass odorized air through the head of a sheep.

The second head was sealed in the same manner as the first. Air at 11 c.c./sec was blown by the variable speed air-blower, *A* (Fig. 1), through the Rotameter flowmeter, *B*, through the bottle, *C*, which in later experiments contained the odorant material, up one nostril of the sheep's head, *D*, and emerging from the other nostril was measured by displacement of water from a cylinder (not shown) which was provided at *E*. In a 5 sec run the air displaced 60 ml. water, corresponding to an air-flow of 12 c.c./sec which was in reasonable agreement with the flowmeter reading of 11 c.c./sec. It showed, moreover, that all the air being blown was actually passing through the nose. Although rubber tubing was used in the train up to the odorant bottle *C*, thereafter to the head (*D*) and in the outlet connexion to *E* polyethylene tubing was used as being less likely to adsorb odorants itself. The arrangement worked best with the head lying on its back; if it was the right side up trouble was experienced with obstruction of the air-flow. Consequently, all the trials were made with the head inverted.

The same apparatus was used for the smell tests; the observer's nose (instead of a water-displacement equipment) was applied to the outlet tube, *E*, to observe the first appearance of smell.

The first experiment was made by blowing the inlet air over benzaldehyde (15 ml.) in bottle *C* at a speed of 10 c.c./sec and observing when the odour of benzaldehyde was first recognizable at the outlet tube *E*. The head was then flushed with air by omitting the odorant bottle (*C*) from the

train and blowing air at 10 c.c./sec for 1 min. Then another run was made with an odorant in bottle *C* and the time taken for the odorant to be recognizable at point *E* was measured; then the head was flushed with air again, another measurement with an odorant was taken, and so on. Three observations were made for each odorant.

RESULTS

Passing odorous air through column of tissues

This method in which air that had been passed over benzaldehyde, and which accordingly smelt strongly of almonds, was passed from the bottom up a vertical adsorption tube packed with chopped tissue, was not satisfactory. Although when the air-blower was switched on and the nose applied to the outlet end of the adsorption tube, the first momentary impression was that the issuing air was odourless and that the chopped material from the olfactory region had adsorbed the benzaldehyde from the odorized air, yet this impression was rapidly dissipated, for in less than a second the almond odour was perceived clearly, so quickly that no certainty could be felt in the initial period of odourlessness. Similar results were obtained with pigmented turbinated material from both sheep and bullock. The small portions of pigmented material proved to be unable to adsorb the bulk of odorant passed through them, although there was some indication in the delay, short though it was, of the smell emerging that some sorption or removal process was at work.

Deodorization of small fixed volumes of air

When benzaldehyde-odorized air had been fed into an empty bottle, the almond smell was found to persist strongly for 10 min, whereas the corresponding bottle to which a large part of the pigmented turbinate from one side of a sheep's head had been added was almost odourless after 30 sec. When, instead of benzaldehyde, there was used air odorized with oil of lemongrass, with methyl octine carboxylate, with triethylamine, or with acetone, very similar results were obtained; ammonium sulphide behaved rather differently and was not rapidly removed from the air by the tissues. The detailed experimental results are shown in Table 1.

In Table 1, the third column headed 'Characterization of odour' gives the characteristic numbers for adsorption on carbon, silica gel, alumina, fuller's earth and vegetable fat as described by the author in an earlier paper (Moncrieff, 1954). For example, the numbers 2 1 3 2 6 for methyl octine carboxylate indicate that it is very readily adsorbed by silica gel (1), readily by carbon (2) and fuller's earth (2), less readily by alumina (3) and only poorly by vegetable fat (6). The sign '−' indicates not that there was no smell, but that no observation was made.

The results included in Table 1 were made on parts of the olfactory membranes removed from five sheep; all of them were lambs about 6–8 months old and either crossbreds or Suffolks. The heads were obtained within a few minutes of the animals having been slaughtered and in two cases

observations on odour removal were made within 45 min of the animal's death. It was surprising to find that the activity of the olfactory membranes so far as smell removal was concerned, seemed to be unimpaired on the day following death; at 2 days they were useless but up to 24 hr their performance was indistinguishable from newly killed. The experiments were made during a spell of cool weather and so as to retard degradation of the specimens the laboratory was not heated, so that its temperature never rose above 50° F. Each one of the observations recorded in Table 1 was made on at least two heads; no considerable differences were noted between the properties of the different heads.

TABLE 1. Sorptive effect on tissue from olfactory receptive region

			Smell of odorized air after retention in					
			Control bottle (no olfactory membrane) for time of			Bottle containing portion of sheep's olfactory membrane for time of		
Air odorized with	Type of smell	Characterization of odour	½ min	2 min.	10 min.	½ min	2 min	10 min
Benzaldehyde	Almonds	0 2 7 1 6	Strong	Strong	Strong	Faintly perceptible	None	None
Oil of Lemongrass	Lemon-like aromatic	4 2 4 4 6	Strong	Strong	Medium	Just perceptible	None	None
Methyl octine carboxylate	Violets	2 1 3 2 6	Strong	Strong	—	None	None	—
Triethylamine	Fishy ammoniacal	3 5 6 5 9	Strong	Strong	—	Clear	Weak	—
Ammonium sulphide	Repulsive irritant	—	Strong	Strong	—	Strong	Strong	—
Acetone	Characteristic	4 3 7 8 9	Strong	Strong	Strong	Clear	Weak	None
Composite 'kitchen malodour' based on furfuryl mercaptan and dimethyl sulphide	Unpleasant cooking odours		Strong	Strong	—	Clear	Clear	—

Smell of grass removed. In the experiment with bottles half full of freshly cut grass there was a great difference observed due to the addition of a portion of the olfactory receptors to one of the bottles. After ½ min it was difficult to be sure if the grass in this bottle could be smelled; probably the odour was somewhere near the threshold value. After 2 min there was no trace of odour, whereas in the control bottle (no membrane) the grass odour was still very strong after 1 hr. At this stage the portion of the olfactory membrane was removed from the test bottle and both bottles, now containing only grass, were left overnight; the following day the control grass still had a faint verdant smell, but the grass in the other bottle, which had originally contained also a portion of the olfactory membrane, was still odourless.

A small number of trials were made with parts of the olfactory membrane from the heads of two wild brown rabbits; these membranes also would rapidly deodorize grass, but unexpectedly did not deodorize air containing benzaldehyde, although they would deodorize air containing methyl octine carboxylate readily enough.

Location of sites of sorption. When pieces of tissue taken from various parts of a sheep's head were used as deodorizers of small volumes of odorized air in beakers it was found that freshly cut tissue from any part of the head, even,

for example, from the chin, had some adsorbent properties, doubtless due to its fineness of surface structure, but that the tissue cut from the pigmented portion of the posterior turbinates excelled in the speed at which it would deodorize air. It can be shown from Table 2 that whereas in the presence of chin tissue or active carbon the smell of benzaldehyde persisted for longer than 1 min, and so it did as a rule in the presence of tissue from the anterior turbinates, yet when a part, even a small part, of the pigmented epithelium was present, the smell was removed in a matter of 25–30 sec. Under experimental conditions such as these, where diffusion of the odorized air in the

TABLE 2. Persistence of odour of benzaldehyde or ethyl acetate in a 600 ml. beaker with various adsorbents. Each figure is the time in seconds at which the odour was no longer detectable

	Benzaldehyde		Ethyl acetate	
Adsorbent	Observer R. W. M.	Observer S. L.	Observer R. W. M.	Observer S. L.
None	220	200	220	240
0·6 g active carbon (Sutcliffe Speakman and Co. Ltd. 207 C) 6–8 mesh	75	150	—	—
0·6 g active carbon Darco 4–12 mesh	75	65	110	122
0·6 g active carbon Darco powder S 51	—	—	95	90
1 g tissue from chin	80	90	130	115
1 g tissue from tip of tongue	60	75	85	80
1 cm^2 anterior turbinate (5 cm from snout)	55	45	101	95
1 cm^2 anterior turbinate (10 cm from snout)	70	55	105	85
1 cm^2 pigmented epithelium from upper posterior turbinate	25 32 22 30 26	32 27 40 28 31	32	52

beaker is slow, one cannot look for very short times of odour removal. The difference, however, between the olfactory epithelium and the other tissues in their deodorizing ability is very marked. The sorptive properties for odorant materials of the olfactory receptors are unique in quality, even although large areas of the nose possess less effective sorptive properties. One result that helped to correlate the presence of pigment with sorptive properties was that in one head it was observed that the pigment in the epithelium extended for some way down the median septum; a little of this skin was removed and was found to deodorize the air in a beaker in about 20 sec, whereas unpigmented skin taken from a nearby portion of the median septum required well over 1 min to effect similar deodorization.

Very similar results were obtained when ethyl acetate was used as the odorant; once again the pigmented epithelium was found to remove the

odorant from odorized air with unique speed. Detailed results are included in Table 2.

The relative shortness of the time of persistence of the smell in the absence of any adsorbent, i.e. in the control beaker, is due to the frequency with which observations were made, the beakers being uncovered to allow each test to be made.

Flow of air through whole head

When the air odorized with benzaldehyde was passed into one nostril of an inverted head (sheep) the air issuing from the other nostril was at first odourless. Despite what had been seen in the earlier experiments with parts of the olfactory membrane cut out and used as adsorbents, it had been expected that sufficient of the benzaldehyde in the air stream would have found its way through the head in a matter of 2 or 3 sec to be smelt at *E*. In fact, when the air had been blowing for 10 sec, no benzaldehyde odour had been detected, and the inlet to the sheep's nose was then disconnected so that the inlet air could be smelled; there was no doubt about it, it smelt very strongly indeed of benzaldehyde. The apparatus was reconnected, but it was another 30 sec before the benzaldehyde odour appeared at the outlet tube, *E*. When this experiment was repeated, after having first flushed out the head by blowing fresh air through it, it again took about 40 sec for the smell of benzaldehyde to be detectable in the issuing air. Various other odorant materials were used and the times required for the emergence of their odour in the outlet air from the nostril was observed. For each odorant at least three observations were made and the spread of these is indicated in the range shown in Table 3. Some smells such as those of phenyl acetylene, onions and amyl acetate came through in a matter of 5 sec; others such as ammonia, camphor and benzaldehyde took about 45 sec.

Sometimes, as with ammonia, the smell emerged sharply and clearly; so it did and in a much shorter time with phenyl acetylene, amyl acetate, and pyridine; with benzaldehyde and methyl octine carboxylate the emergence was much more gradual and it was less easy to fix a point at which the odour was first recognizable. It is, however, quite clear from Table 3 that some of the odorants are withdrawn from the odorized air in their passage through the nose, and furthermore that the sorptive powers of the sheep's nose vary very greatly from one kind of odorant to another.

The experimental results recorded in Table 3 were made on three different heads, all of Suffolk lambs, and all within 26 hr of death. Performance some 24 hr after death did not differ noticeably from that of 1 hr after death. No observations were made later than 26 hr after death. All three heads gave very similar results.

Because the time of recognition of odorant material at the outlet of the nose varied from 4 sec to more than 60 sec, and because the rate of air-flow was throughout from 10–12 c.c./sec, a volume of from 40 to 700 c.c. of odorized air

passed through the head before the odour was recognizable. It seemed to be of interest to relate this to the internal volume of the air space in the head. Accordingly, one head was filled by gently blowing water into it until the water emerged from the other nostril. In this way it was found that the internal volume of the head was 98 ml. and that of the tubing leads was 10 ml. So that if there were neither eddy currents nor air turbulence it would take some 10 sec for air blown into one nostril to emerge from the other. Evidently, eddy currents and turbulence do occur, as, indeed, they would be expected to in the

TABLE 3. Times of sorption when odorized air is passed at 10 c.c./sec through the nasal passages of a sheep's head

Air odorized with	Type of smell	Characterization of odour	Time (sec) taken for smell to be recognized at outlet tube
Benzaldehyde	Almonds	0 2 7 1 6	35–50
Dioxan	Ethereal	2 2 5 2 7	30–40
Methyl octine carboxylate	Violets	2 1 3 2 6	10–16
Oil of Lemongrass	Lemon-like aromatic	4 2 4 4 6	6–11
Fresh grass	Vernal, fresh, sweet	—	>60
Fresh catmint	Characteristic	—	>60
Freshly cut onions	Characteristic	0 3 7 8 9	5–6
Ammonia (15% w/w in water)	Characteristic	—	37–47
Phenyl acetylene	Coal gas	2 4 7 6 9	4–5
Amyl acetate	Pear drops	—	5–7
Camphor	Characteristic	—	50–60
n-Butanol	Spirituous, bitter	0 1 4 0 9	12–17
Pyridine	Rank, repulsive	2 2 6 6 8	10–12

flow of air through turbinated channels. On one occasion the smell of phenyl acetylene was unmistakably observed in the outlet air only 4 sec after it had been introduced into the inlet. On the other hand, the finding that ammonia, camphor and benzaldehyde took 40 or 50 sec to find their way through suggests that sorption must have been very effective in these cases, because in this time all the air in the head would have been completely changed some four times.

Quantity of odorant adsorbed. In order to find what quantity of odorant material was being removed from the air by the sorptive action of the interior nasal surfaces, an estimation was made on the air odorized with benzaldehyde. This air was blown directly at a speed of 10 c.c./sec into 50 ml. N/1000 $KMnO_4$ (0·0316 g/l.) which had been acidified with sulphuric acid, until the air decolorized the permanganate. This required 8 min 30 sec. Hence, about $5\mu g$ (3×10^{16} molecules) of benzaldehyde was blown every second. At first, the air that bubbled out through the permanganate was odourless, but as the end-point was approached the benzaldehyde odour could be detected. In the 40 sec that it required for the odour of benzaldehyde to pass through the sheep's head some 10^{18} molecules of benzaldehyde must have been passed in

and most of them must have been adsorbed, because no benzaldehyde could be smelt in the effluent air until after the expiration of 40 sec.

Reversibility of sorption

Some results indicated that the sorptive process was reversible. For example: air was passed at 10 c.c./sec over oil of lemongrass and then through the sheep's head; the emergent air was smelled and at 9 sec the odour of lemongrass was first perceived; at 11 sec it was strong and the air-blower was stopped. The air feed to the head was disconnected and reconnected so that fresh air was blown into the head, the odorant bottle, *C*, being by-passed. This change took 20–30 sec to make and then the air-blower was re-started. The odour of lemongrass was unmistakable in the emergent air at first but quickly weakened and could not be smelled after about 2 sec. There was, nevertheless, a positive indication that the sorptive process was at least partly reversible.

Onion. Air was passed at 10 c.c./sec over freshly cut onion and then through the sheep's head. The smell of onion appeared clearly at 5 sec and the air-blower was then stopped. The inlet leads were changed so that the odorant bottle, *C*, was cut out and the blower was re-started. For the first $1\frac{1}{2}$–2 sec the odour of onion was quite strong in the emergent air, thereafter it was lost but one soon loses track of a rapidly weakening odour.

Phenyl acetylene. This was used similarly; it was perceived at 4–5 sec very strongly. When inodorous air was subsequently passed in, the odour of phenyl acetylene was perceptible in the emergent air for 40 sec.

Pyridine. This was perceived after 10 sec. When fresh air was subsequently passed it smelled strongly of pyridine, not just for a second or two but even after 60 sec. Suspecting that the polyethylene tubing to and from the nostrils might have adsorbed some pyridine and was still giving it up slowly, these leads were replaced by new ones, but on re-starting the blower the smell of pyridine appeared at once and persisted for 300 sec. This pyridine was certainly coming from the sheep's head. The reappearance and persistence of smell when fresh air was blown through was not simply due to displacing in the nasal cavities the air, which would have contained the odorant if the adsorption process had reached saturation; if it had been so the time of persistence would have been approximately the same whatever odorant was used. In practice it varied from 2 sec with onion to 300 sec with pyridine.

Amyl acetate. This was run for 7 sec until it smelt very strong; the blower was stopped and the apparatus left overnight. The next morning air was blown through to flush out the head and the odour of amyl acetate emerged strongly. There was, therefore, evidence of the reversible character of the sorption on the olfactory membrane of lemongrass, onion, phenyl acetylene, pyridine and amyl acetate.

Displacement of one odorant by another

Another phenomenon that was observed at first by chance, during these experiments, and which also points to the sorption being a reversible process, was that of one odorant being displaced by another. Methyl octine carboxylate and acetone seemed to be particularly liable to such displacement. Air odorized with methyl octine carboxylate had been passed through a sheep's head to the point of recognition; thereafter inodorous air had been passed through for 3 or 4 min to flush out the head thoroughly, and this flushing air was (after the first few seconds) quite odourless on emergence. Next, air odorized with ammonia was passed through the head; it was some 40 sec before the smell of ammonia was recognizable but in that interval the smell of methyl octine carboxylate was clearly recognized in the emergent air.

An attempt was made to see if such displacement would work in reverse. Ammonia was passed through the head; it was detected at 40 sec but flow of air odorized with ammonia was continued for 90 sec in all. Then the head was flushed with air; even after 12 min at 10 c.c./sec the emergent air would blue B.D.H. Universal test paper rapidly; the air-flow was increased to 20 c.c./sec and after another hour of flushing with air the emergent air took about 6 sec at 20 c.c./sec to blue a damp test paper (the inlet air to the head would not blue a test paper at all). The air speed was reduced to 10 c.c./sec and a time of 15 sec was required for the emergent air from the head to blue the test paper. Then the blower was stopped and air odorized with methyl octine carboxylate was blown through the head at 10 c.c./sec; the emergent air would now blue a test paper in 6 sec. On another occasion amyl acetate had been used and the sheep's head subsequently well flushed. Then air odorized with camphor was passed and before the camphor smell emerged, the amyl acetate smell again came through strongly and unexpectedly.

DISCUSSION

The rapid deodorization by parts of the turbinated material from the heads of sheep, bullocks and rabbits of air odorized with benzaldehyde and similar compounds, shows that some sorptive process is taking place; the easy susceptibility of the smell of newly cut grass to such sorption suggests that the process is one which normally occurs in the living animal. When it was found that the posterior turbinates which carry the pigmented olfactory epithelium were unique in the speed with which they promoted deodorization, a close link between the smell receptors and the ability to remove odorant molecules from odorized air was established. All the internal tissues, by virtue of their fineness of structure and their complexity of surface structure, exhibit sorptive properties, but these, good as they are when compared with some inorganic

adsorbents, are much inferior to those possessed by the epithelium in the olfactory region.

It was at first surprising to find when odorized air was passed through the nasal passages of a dead animal that the odour was removed entirely; in retrospect the indication that there is a very efficient and rapid concentration process taking place at the expense of the air passing through the nose, is exactly what we might expect to find associated with the sense of smell, with its extreme sensitiveness to the most tenuous of vapours and with its apparent instantaneity of response. Even so, the efficiency and rapidity of the process that completely deodorizes strongly smelling air was quite startling when first encountered. It was, too, surprising to find that the heads of animals which had been dead for some 24–30 hr had sorptive properties that were substantially the same as when the animals had been dead only some 20 min; probably this behaviour is an indication that the sorptive process that goes on is a *physical* rather than a biochemical process. The physical nature of the process of deodorization of air in the nose was also plainly indicated by the way in which the same head could be used repeatedly with different odorants, with intermediate flushings out, and apparently with properties that were unchanged over a long series of experiments; so far as concerned its deodorizing properties, the head behaved more like a physical instrument than a physiological specimen, doubtless because the requirement made of it was one that was simply physical—that of adsorbing odorants. Furthermore, the experimental results showed clearly that the sorptive process was at least sometimes reversible, and also that preferences of sorption were sometimes shown, that after one substance had been taken up by the olfactory epithelium, it could be turned out on the arrival of another substance. Both reversibility and preferences are characterisic of the physical process of adsorption. There is no doubt that the odorant molecules are removed by the interior nasal surfaces, and mainly by the olfactory epithelium in the passage of odorized air through the nose, and there are certain pointers (slowness of decay of powers of concentration, rapidity of and completeness of action, reversibility and exhibition of preferences) to this removal or concentration process being nothing more nor less than one of physical adsorption.

The mechanics of adsorption

In order to correlate the experimental results with both adsorption and the properties of smell, it is helpful to have a mind picture of the *dynamic* nature of adsorption. When a gas molecule collides with a solid surface and bounces off with the angle of reflexion equal to the angle of incidence, only pressure, e.g. that of the atmosphere, is exerted. But some kinds of solid surfaces have an attractive force for some kinds of gas molecules and hold them momentarily until the vibrations of the surface atoms impart sufficient energy to the captured

gas molecules for them to fly off back in any direction into the gas. It is the *stay*, short though it is, which is important; it means that a *concentration* of gas molecules is built up on the surface of the solid, or that the surface has sorptive properties for the gas. The longer the stay of the gas molecule on the solid surface, the greater will be the concentration of the gas absorbed. According to de Boer (1953): 'Heats of adsorption between 10 and 15 kcal/mole represent figures found for many gases consisting of heavier molecules, including the molecules of many organic substances, at various technical adsorbents.' Such heats of adsorption would correspond to times of adsorption (i.e. average time of stay of the gas molecules on the solid surface) of about 10^{-6}–10^{-2} sec and if an organic odorant molecule makes a stay of 10^{-6} sec there will soon be an enormous concentration of such molecules on the adsorbent surface. If about 10^{23} of them hit each cm^2 of the olfactory receptors every second, and if their time of stay, i.e. their time of adsorption is 10^{-6} sec, then at any moment there will be $10^{23} \times 10^{-6}$ or 10^{17} molecules adsorbed on each cm^2 of surface (or whatever smaller number is sufficient to complete a monomolecular layer). If an odorant is mixed in air in a concentration of 0·01 %, then there will, with a time of adsorption of 10^{-6} sec, soon be $10^{23} \times 10^{-4} \times 10^{-6}$ or 10^{13} molecules of odorant adsorbed per cm^2 of solid surface. It is apposite to recall that the pigmented olfactory membrane in man extends to an area of 5 cm^2 on either side. Equilibrium is rapidly reached, but there is no rest; each millionth of a second millions of millions of molecules are touching down on to the solid surface and an exactly equal number are taking off. The system is dynamic. It will be of interest to examine the implications of the quantitative measurement of one odorant which was picked up by the experimental sheep's head.

Quantity of benzaldehyde adsorbed

The quantity of benzaldehyde picked up by the interior nasal surfaces of a sheep was found experimentally to be 200 μg or 10^{18} molecules. If we postulate that these molecules are arrranged in a monomolecular layer we can calculate that they would cover an area of about 3 m^2. At first sight this area seems to be very much larger than anything we could expect to find in the head of a sheep, and furthermore, if smell is stimulated by adsorption and if quality discrimination is due to selectivity of adsorption it would seem likely that only a portion of the surface adsorption sites would be filled by any particular adsorbate, so that we may have to recognize an area of 3 m^2 as being only a *part* of that available for olfactory stimulation.

There are two possible explanations:

(1) That the true surface area of the olfactory membrane is enormously greater than the apparent area from inspection would suggest. There is nothing unlikely in this. All of the common industrial adsorbent materials have

enormous area/mass ratios, because they are so riddled with cavities, fissures and pores; thus fuller's earth can have a surface area of 80 m^2 per gram according to Granquist, Mitch & Edwards (1954) and even higher ratios are to be found in activated carbon. An area of 3 m^2 would be given by about 0·04 g of fuller's earth. There is no reason to suppose that Nature, in choosing adsorption as the process that would enable primitive forms of life through their ability to detect, by the sense of smell, food and mate, to survive at all, would choose an adsorbent inferior in quality to those that we so easily make by the hundreds of tons. Very possibly the adsorbent in the olfactory areas will set us some quite new standards for adsorbents.

(2) That the adsorption is not mono- but multi-molecular, that the layer of odorant adsorbed on the olfactory membrane may be perhaps a hundred molecules thick.

Until we know more about the real area of the olfactory epithelium there is insufficient evidence available to choose between the two possibilities, although the behaviour of the sense of smell points to the first as being the more likely. Some support for this view comes, too, from the work of Bloom & Engström (1952) and Engström & Bloom (1953). They have found by electron-micrography of the olfactory region that its surface is covered by an enormous number of hair-like filaments by means of which 'the olfactory region acquires an enormous enlargement of its free surface, which must be of the utmost importance for its physiology'. It can be calculated from their results that even if the filaments have *smooth* surfaces, which seems unlikely, their total area in man is of the order of 0·1 m^2; if their surfaces are wrinkled, pitted or fissured then their area will be correspondingly greater.

The smell stimulus

It is a matter of everyday experience that odour is air-borne; we smell downwind, and we cannot smell through any air barrier such as a glass bottle. It is generally agreed (Dyson, 1938) that a prerequisite for a substance to be odorous is that it shall be volatile; no non-volatile substance is odorous. The primary stimulus for olfaction is the presence of molecules of an odorant material in the air which is inspired and is drawn over the olfactory membrane. What happens when the odorant molecules reach the olfactory receptors is not as yet generally agreed. The possibilities would seem to be: (1) simple contact of odorant and receptor; (2) a chemical reaction between odorant and receptor; (3) solution, i.e. the odorant dissolves in the tissues of the receptors; (4) adsorption of the odorant on the receptor surfaces.

Simple contact of the odorant molecules with the receptors simply means that the odorant gas molecules hit the receptor surfaces and bounce off immediately; this would do no more than exert pressure, and the pressure would be the same whether pure air or odorized air was inspired. It is *change*

that is necessary for stimulus and such change would not be provided by the odorant molecules if nothing more than simple contact was involved.

The sort of chemical theory that might be put forward is that the olfactory cell would contain enzymes capable of inducing a reaction between the odorant and some other material in the cell. The reaction would have to take place rapidly and the end products would presumably be removed by the blood stream. A considerable objection to any theory of this kind is that many odorant materials are fully saturated and chemically unreactive. For example, hexane and heptane and similar fully saturated hydrocarbons are strongly odorous but they are unlikely to take part in any chemical reaction under the conditions of temperature and pressure that obtain in the body.

The possibility that the olfactory stimulus is simply the act of solution of the odorant in the nasal liquids has received some attention. Backman (1917) has suggested that both aqueous and lipoid solubility are necessary for smell, and he has instanced the homologous series of aliphatic alcohols; methyl and ethyl alcohols are both readily soluble in water but they have only low solubilities in fatty substances and they are, therefore, only mildly odorous, whilst the high members of the series such as cetyl alcohol, although readily fat-soluble, are insoluble in water and therefore inodorous. Intermediate members such as the butyl and amyl alcohols are soluble in both water and fats and therefore have powerful smells. Such considerations, however, must be restricted to a homologous series, otherwise chemical differences will far outweigh solubility differences; for example, acetone has only a low solubility in lipoids but it has a strong smell, and xylene and naphthalene, which are practically insoluble in aqueous liquids, both have strong smells. McCord & Witheridge (1949) have suggested 'on the basis of frankly labeled speculation' that the odorant dissolves in the nasal equipment and that the bonding angles which unite the atoms in the odorant molecule are modified during solution and that it is this modification that provides the stimulus. Undoubtedly there are parallels that can be drawn between smell and solubility, as indeed there are between smell and almost any physical property; probably that is as far as it is safe to go. The main difficulty in the way of accepting solution as the smell stimulus is the insolubility of many powerful odorants in the body fluids.

The fourth possibility, that it is adsorption of the odorant molecules on the olfactory membrane that provides the stimulus for olfaction, does not seem to suffer from such serious disadvantages. Furthermore, adsorption is a rapid process in which equilibrium may be established in a hundredth or a thousandth part of a second; it is a completely reversible process; it is 'clean' in the sense that there are no chemical residues to dispose of; and always the adsorption of a gas on a solid surface is, because of the loss of freedom of the gas molecules, exothermic so that energy is released. There is also the evidence given in an

earlier paper (Moncrieff, 1954), that substances with similar smells behave similarly amongst themselves to different adsorbents, but differently from other substances with different smells.

It may, too, be significant that those adsorbents that are most useful such as charcoal and silica gel will very quickly and often completely adsorb odorous material from air—they deodorize it. The one process, from all chemical and physical processes with which we are familiar, which is most closely associated with smell is adsorption. None of the others: boiling, condensing, melting, dissolving, crystallizing, illuminating, irradiating, electrifying, magnetizing, oxidizing, reducing, hydrolysing, polymerizing, has that special connotation with smell that adsorption has. The easiest way to catch a smell and hold it for future reference is to adsorb it.

The experimental findings given earlier in this paper, that odorous air can be deodorized by the interior nasal passages of a dead animal, show clearly that sorption of the odorant does take place and there are strong indications that the sorptive process is one of physical adsorption. The sorptive behaviour of portions of the olfactory membrane, when isolated, affords supporting evidence that adsorption is the initial step in the stimulation of smell.

The dynamic character of adsorption accounts satisfactorily for the apparent instantaneity of smell perception and for its equally rapid cessation when the odour source is removed. The same dynamic character accounts easily for our ability to discriminate between similar odours of different intensity because when equilibrium is reached between those odorant molecules that have been adsorbed on the olfactory surfaces and those that are in the air in the nasal passages, then the area of receptor surface covered by adsorbate molecules will be greater, the higher is the concentration of odorant molecules in the inspired air. Quality discrimination of smell is also easy to envisage; it will depend on whichever areas of the receptors are covered with a molecular layer of adsorbed gas molecules. Adrian's (1954) work shows that there is a specificity of spatial activation; doubtless there are different kinds of receptors, some favourable to lodgment of molecules of spherical shape which, according to Timmermans (1954), will often give a camphoraceous type of odour, whilst others will afford suitable sites for the adsorption of different kinds of molecules with different shapes. Additionally, the time of adsorption, i.e. average time spent by the molecules on the receptors, is a characteristic which alone could account for an infinity of smells; all the evidence shows that it varies greatly from one gas to another, that it is characteristic for a particular substance on a given adsorbent. It is possible that the temporal characteristics may be as important as the spatial in determining the quality of a smell.

Finally, unless there were some selective process such as adsorption which could concentrate the odorant molecules, it is very difficult to imagine how

such immense dilutions in air of odorants like the mercaptans and the musks can stimulate smell, as they are known to do. The fundamental feature of adsorption is that it is a process of *concentration*.

SUMMARY

1. It is shown that the olfactory membrane when cut away from a newly killed animal will render some kinds of odorized air odourless.

2. It is also shown that when odorized air is passed through the nasal passages of the sealed head of a newly killed animal it emerges odourless, for a time which depends on the nature of the odorant.

3. The implications of these sorptive properties of the olfactory membrane are examined and are correlated with the properties of smell.

The work described has been carried out with funds provided by Airkem Inc., New York.

REFERENCES

ADRIAN, E. D. (1954). The basis of sensation: some recent studies of olfaction. *Brit. med. J.* i, 287–290.

BACKMAN, E. L. (1917). Note sur la puissance des odeurs et leur solubilité dans l'eau et dans l'huile. *J. Physiol. Path. gen.* **17**, 1–4.

BLOOM, G. & ENGSTRÖM, H. (1952). The structure of the epithelial surface in the olfactory region. *Exp. Cell Res.* **3**, 699–701.

DE BOER, J. H. (1953). *The Dynamical Character of Adsorption*, p. 36. Oxford: Clarendon Press.

DYSON, G. M. (1938). Scientific basis of odour. *Chem. & Ind. (Rev.)*, **57**, 647–651.

ENGSTRÖM, H. & BLOOM, G. (1953). The structure of the olfactory region in man. *Acta oto-laryng., Stockh.*, **43**, 11–21.

GRANQUIST, W. T., MITCH, F. A. & EDWARDS, C. H. (1954). Adsorption of normal saturated hydrocarbons on fuller's earth. *Industr. Engng Chem.* **46**, 358–362.

MCCORD, C. P. & WITHERIDGE, W. N. (1949). *Odors: Physiology and Control*, pp. 22–23. New York: McGraw-Hill.

MONCRIEFF, R. W. (1954). The characterization of odours. *J. Physiol.* **125**, 435–465.

TIMMERMANS, J. (1954). Odour and chemical constitution. *Nature, Lond.*, **174**, 235.