Equol, first isolatedfrom equine urine in 1932 and identified 50 years later in human urine as ametabolite of the soy isoflavones, daidzin and daidzein, is produced byintestinal bacteria in some, but not all, adults. This observation led to theterm equol-producers to define those adults that could make equol in responseto consuming soy isoflavones and the hypothesis that the health benefits ofsoy-based diets may be greater in equol-producers than in equol nonproducers.By virtue of a chiral center, equol occurs as a diastereoisomer and intestinalbacteria are enantiospecific in synthesizing exclusively the S-(-)equolenantiomer, an enantiomer that has selective affinity for the estrogenreceptor-b. Both enantiomers are of interest from a clinical andpharmacological perspective and are currently being developed as nutraceuticaland pharmacological agents. The wide range of biological activities theseenantiomers possess warrants their investigation for the treatment of a numberof hormone-related conditions involving estrogen-dependent and androgen-relatedconditions. The following review describes the history, chemistry, and factorsgoverning the intestinal bacterial formation of equol. J. Nutr. 140: 1355S–1362S,2010. Introduction It is now 57 years since the first report appeareddescribing a new phenolic compound in an estrogenic fraction of pregnant mareurine (1). It was suggested that the compound be given the name equol, afterthe equine source of the material. Efforts to obtain large-scale quantities ofthe compound led to the recognition that it was also present in appreciableamounts in the urine of stallions and nonpregnant mares and the conclusion thatit was not associated with the presence of high estrogen states. During theautumn months, the amounts of equol declined and by winter it was impossible toisolate it from urine. The authors concluded that, “so far as can bedetermined, no dietary factor was the cause of this (seasonal) variation…” (2).It later became apparent that this was not the case when in SWAustralia reportsemerged of a catastrophic “failure to breed” associated with uterineabnormalities and endometriosis in sheep grazing on Trifolium subterraniumclover (3). Reductions in sperm counts and motility were also documented inewes (4). This clover disease, as it was so-called, was found to be the resultof extremely high circulating concentrations of equol, formed by rumenalbacteria from the ingestion of large amounts of the methoxylated isoflavone,formononetin, abundant in several indigent species of clover (5–7). Equol waseven found as a component in urinary calculi of sheep and cattle (8). Equol hassince been reported to be present in the urine and/or plasma of many otheranimal species, including cows (9), hens (10–14), monkeys (15,16), chimpanzees(17,18), dogs (19), mice (20), rats (20–22), and pigs (16,23), but there aremarked differences in the extent of metabolism of isoflavones into equol bythese species. Rodents, e.g., very efficiently convert daidzin/daidzein toequol (24), whereas pigs and humans have been reported to do this lessefficiently (16,24). In the decades leading to 1970, a great deal of work wasperformed defining the metabolism of isoflavones and biological actions ofequol (6,25–28). While its estrogenic effects were well documented based uponfield observations and classical bioassays, it was not until after thediscovery of the first estrogen receptor (ER) in the mid-1960s (29) that therelative affinity of equol for the ER could be 1 Published in a supplement toThe Journal of Nutrition. Presented at the “Equol,

Soy, and Menopause ResearchLeadership Conference", held in Washington, DC, June 16, 2009. The supplementcoordinator for this supplement is Kara Lewis, Life Sciences ResearchOrganization (LSRO) Senior Staff Scientist. The supplement is theresponsibility of the guest editors to whom the Editor of The Journal ofNutrition has delegated supervision of both technical conformity to the publishedregulations of The Journal of Nutrition and general oversight of the scientificmerit of each article. Publication costs for this supplement were defrayed inpart by the payment of page charges. This publication must therefore be herebymarked "advertisement" in accordance with 18 USC section 1734 solely toindicate this fact. The Guest Editor for this supplement is Neil Shay. GuestEditor disclosure: Neil Shay declares no conflict of interest. SupplementCoordinator disclosure: Kara Lewis is currently under contract with andreceives compensation from the supplement sponsor. She was also compensated forattending and organizing the Equol, Soy, and Menopause Research LeadershipConference and for organizing, writing, editing, or reviewing, and collectionof supplemental manuscripts. The opinions expressed in this publication arethose of the authors and are not attributable to the sponsors or the publisher,Editor, or Editorial Board of The Journal of Nutrition. 2 Author disclosures K.D. R. Setchell was supported by funding from the NIH (grant nos. R01AT-003313and R01AT-002190) and has intellectual property on equol enantiomers, includingpatents licensed by Cincinnati Children's Hospital Medical Center, Cincinnati,OH, to industry and is a consultant to Otsuka Pharmaceuticals Company, Tokyo,Japan. C. Clerici, no conflict of interest. * To whom correspondence should beaddressed. E-mail: kenneth.setchell@ cchmc.org. 5 Abbreviations used: BBM,brush border membrane; ER, estrogen receptor; ISP, isolated soy proteins; RMB,relative molar binding. 1355S Firstpublished online June 2, 2010; doi:10.3945/jn.109.119776. quantified. When the relative molar bindingaffinities of a number of phytoestrogens for sheep uterine ER were compared,equol was found to have much higher affinity than its precursor daidzein incompeting with radioactive estradiol for binding to the cytosolic receptor(27), supporting the theory that it may be advantageous to be able to convertdaidzein to equol (24). There was little interest in equol for several decadesuntil the chance discovery in 1980 of high concentrations of an unknownestrogen-like compound in rat urine (30) accompanying the mammalian lignans,enterolactone and enterodiol (31–33). At the time, it was referred to ascompound 386/192, a notation for the molecular ion and base peak in the massspectrum of its trimethylsilyl ether derivative. In common with most endogenoussteroid hormones, including estrogens, it was conjugated predominantly toglucuronic acid and a lesser extent to sulfuric acid (34). Its presence in suchhigh concentrations in rat urine fortuitously afforded a means of isolatingsufficient quantities for structural elucidation studies by infraredspectroscopy, NMR, and GC-MS (35) and the subsequent confirmation that it wasidentical in chemical structure to the equol first isolated from pregnant maresurine in 1932 by Marrian et al. (1,2). This confirmation was made possiblebecause one of us (K.D.R.S.) was gifted from the curator of the UK MedicalResearch Council's Steroid Reference Collection (the late Professor D.N. Kirk)the original 4.0 mg sample of equol

isolated from pregnant mares urine byMarrian et al in 1932. Equol was also found to occur as a minor constituent inthe urine of many adults. The link between equol and soy came about after aseries of studies in which different plant-based foods were fed to ratsmaintained on a purified diet. The introduction of soy protein led to a hugeincrease in the urinary excretion of equol and following this observation, thesoy isoflavone daidzin was isolated and shown to be a precursor to equol (35).It was also found that the introduction of soy protein to the diet led to anincreased excretion of equol in some but not all adults (36), whereas in vitroincubation of cultured fecal flora from equol-producing individuals with eitherdaidzein or soy protein resulted in the formation of equol (36). The finding ofhigh concentrations of equol in the urine of adults consuming soy foodsprompted the hypothesis that this nonsteroidal estrogen may be beneficial inthe prevention and treatment of many hormone-dependent conditions (36).Progress in research studies of equol was hampered by the lack of sufficientamounts of the compound for biological and clinical testing and by thedivergent interest and focus on genistein, the other soy-derived isoflavonethat was shown to be an potent inhibitor of tyrosine protein kinases (37) and acompound that was readily available in bulk. Almost 20 y after the finding ofequol in human urine, it was proposed that the efficiency with which adultsconvert daidzein to equol when consuming diets containing soy foods couldenhance the clinical effectiveness of soy-based diets—the so calledequol-hypothesis (24)—and this has driven a resurgence of interest in equol, asis evident from the almost exponential increase since 1980 in the number ofpublications cited by a PUBMED search of this as a keyword (Fig. 1). At therecent 8th International Soy Symposium held in Tokyo, Japan, for the first timean entire session was devoted to equol (38). This overview will focus on someof the key areas related to this unique molecule and is not intended to be a comprehensivereview of the topic. Chemistry Equol [7-hydroxy-3-(49-hydroxyphenyl)-chroman],an isoflavan, belongs to the general class of compounds referred to asnonsteroidal estrogens. It has a molecular composition of C15H1403 and amolecular weight of 242.27 Daltons. The heterocyclic structure contains 2reactive hydroxyls and 1 relatively inert and unreactive oxygen in the centralfuran ring.

EQUOL : HISTORY , CHEMISTRY , AND FORMATION 1 , 2

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